Cellular Immunotherapy for Cancer

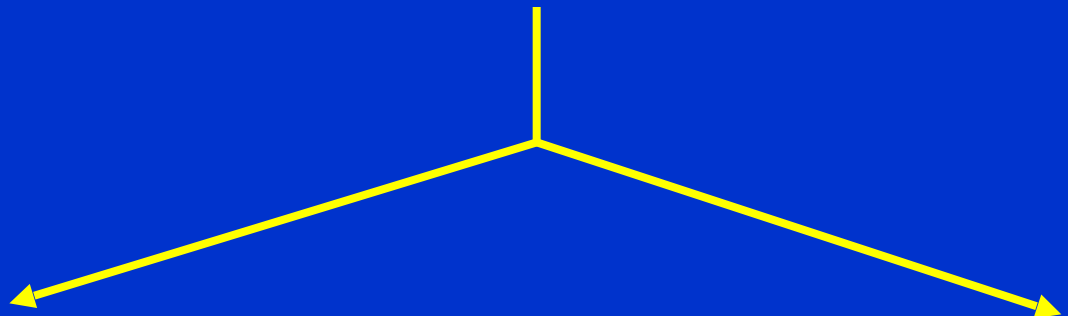
Joseph Skitzki MD, FACS

March 22nd, 2016

Objectives

- explain the rationale behind cellular adoptive immunotherapy
- describe methods of improving cellular adoptive immunotherapy
- identify mechanisms of tumor escape from cellular adoptive immunotherapy

The Immune System



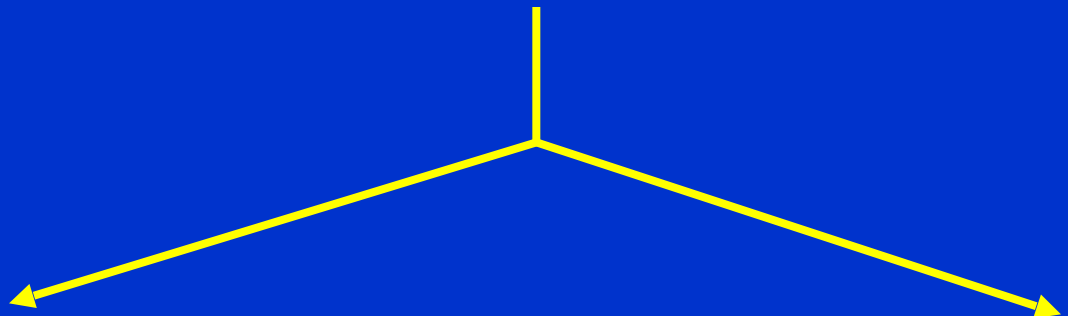
Innate

- skin, mucosal barriers
- complement
- neutrophils, NK cells, mast cells, basophils, eosinophils

Adaptive

- T cell mediated
  - CD8 cytotoxic
  - CD4 helper
- B cell mediated
  - antibody production

Immunotherapy for Cancer



Active ← - - - - - - - - - - - - **Passive**

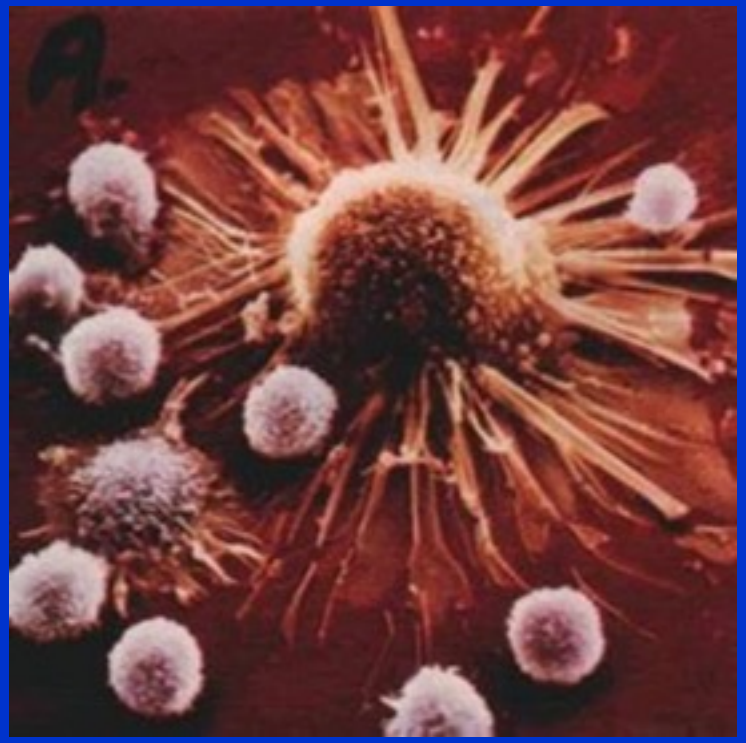
- vaccines

- cell transfer strategies
"Adoptive Immunotherapy"

Cancer as an immune target

Evidence

- unique cancer antigens
- immunosuppression
- "immune editing"[1] –
  - elimination phase
  - equilibrium phase
  - escape phase



[1]Immunity 2004 21(2):137-148

Benefits of Immunotherapy

- relatively non-toxic therapy
- exquisitely specific
- curative/adaptive
- long lived protection/memory

Immunotherapy for Cancer

Passive

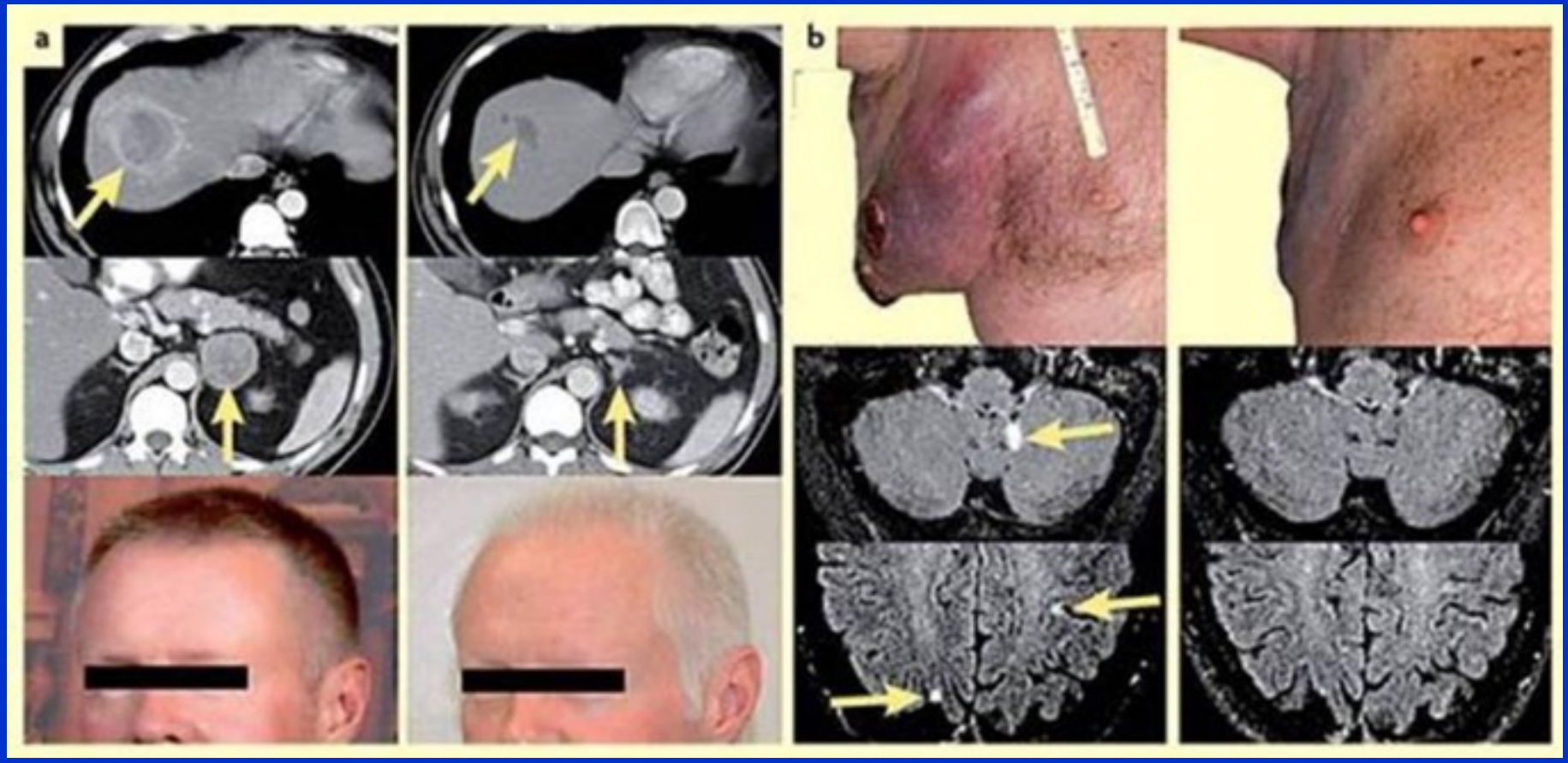
- cell transfer strategies
"Adoptive Immunotherapy"

1960's	1970's	1980's	1990's	2011
early animal models	Immuno-suppression required	IL-2 LAK cells	TIL cells TDLN cells	

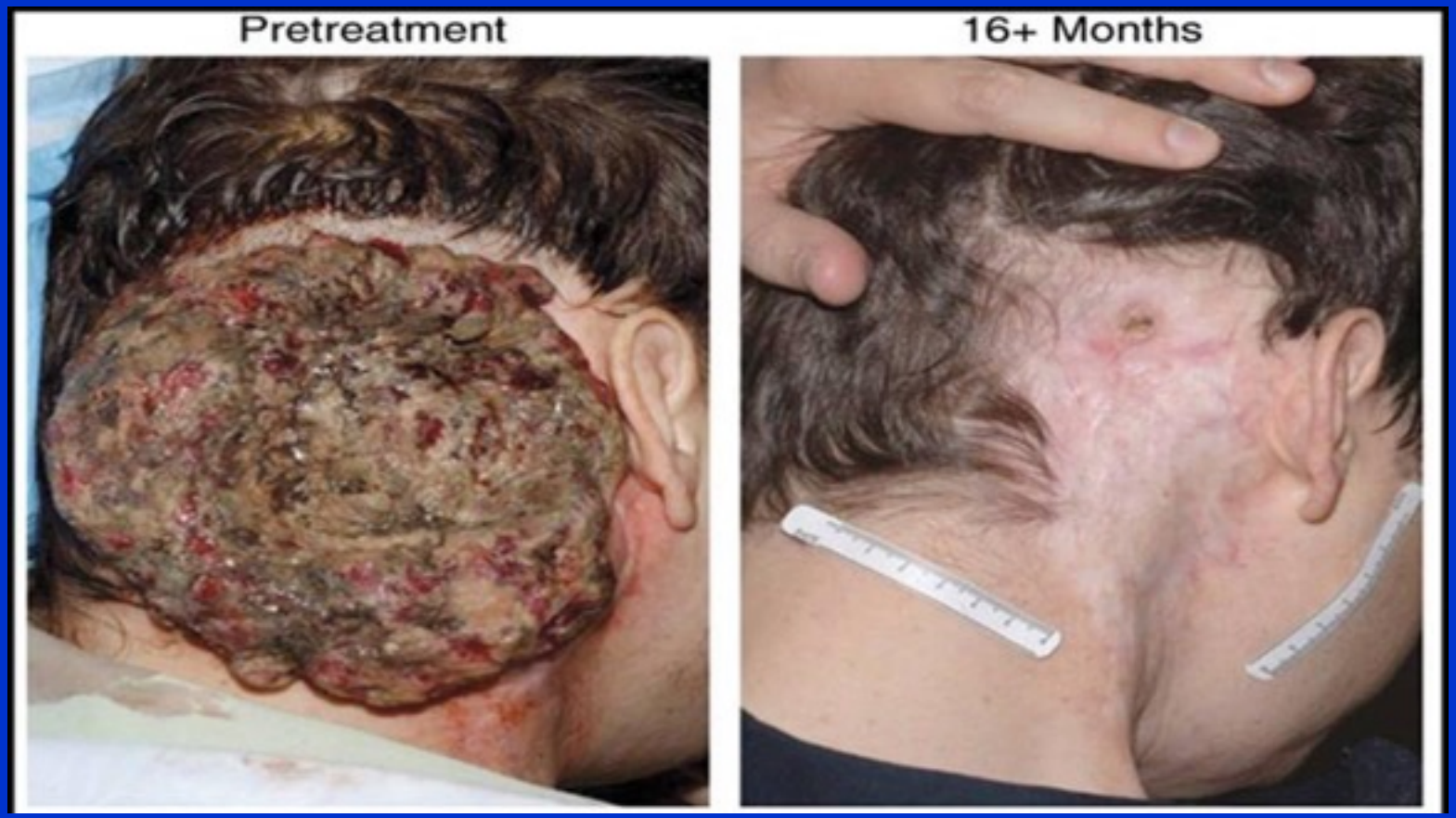
Advantages of Passive Cellular Approach

Pros	Cons
- feasible	- labor intensive
- activation and expansion without dampening factors	- expensive
- supraphysiologic numbers of cells	- too artificial(?)
- ex vivo genetic manipulation of cells	- transient cell viability
	- no ‘off-the-shelf’ ability

Advantages of Passive Cellular Approach

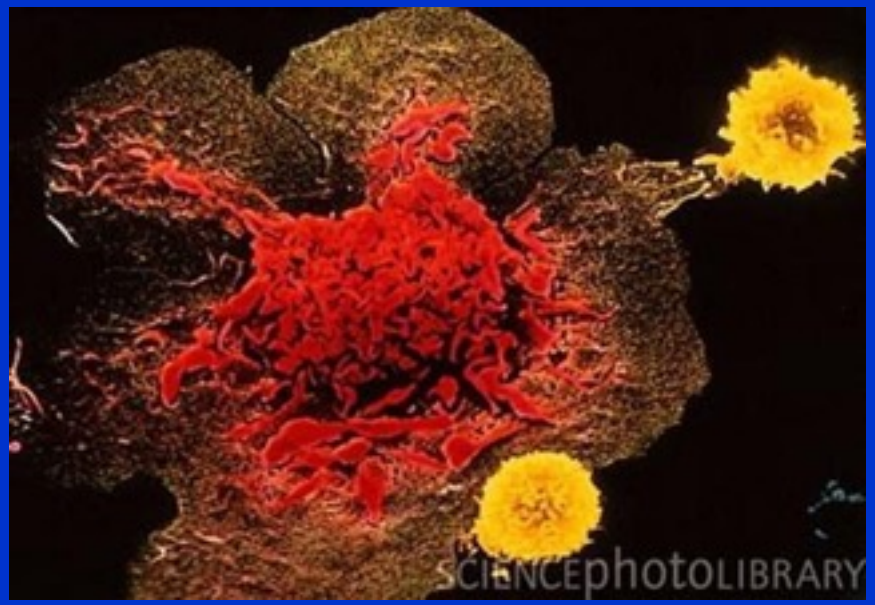


Advantages of Passive Cellular Approach



Immunotherapy for Cancer: 1970-1980

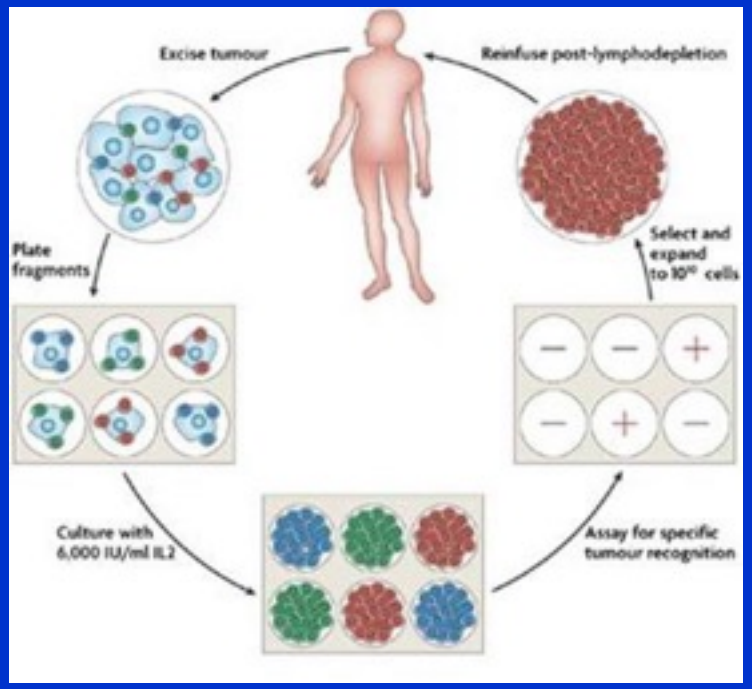
- Rosenberg et al[2]. – cultured mononuclear fraction of peripheral blood (leucopheresis) with IL-2
- generated lymphokine-activated killer cells (LAK)
- LAKs found to be NK cells
- when given with exogenous IL-2 – clinical responses
- improved with cyclophosphamide or TBI[3,4]

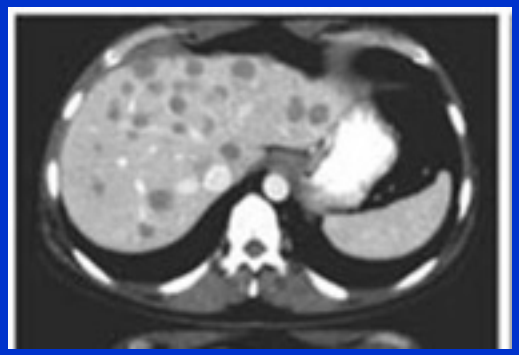


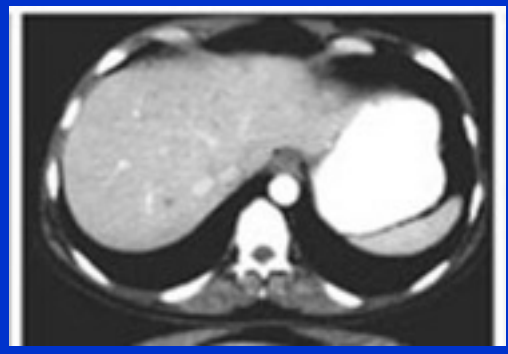
[2]Cancer Res 1981; 41: 4420-4425.
[3]J Immunol 1985; 135: 646-652.
[4]Science 1986; 233: 1318-1321.

Immunotherapy for Cancer: 1980-1990

- Rosenberg et al[5]. – cultured tumor suspension in IL-2
- generated tumor-infiltrating lymphocytes (TIL)
- TILs determined to be mostly CD8 T cells
- when given with exogenous IL-2 – clinical responses
- 50-100x more effective than LAK cells



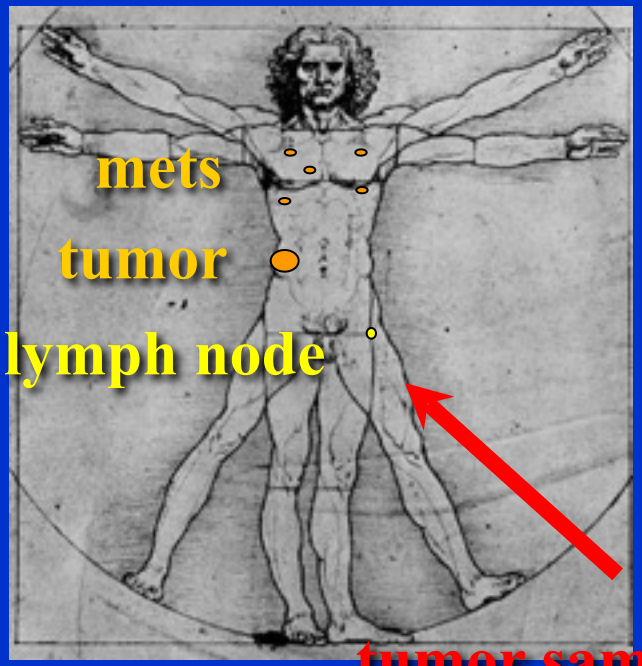


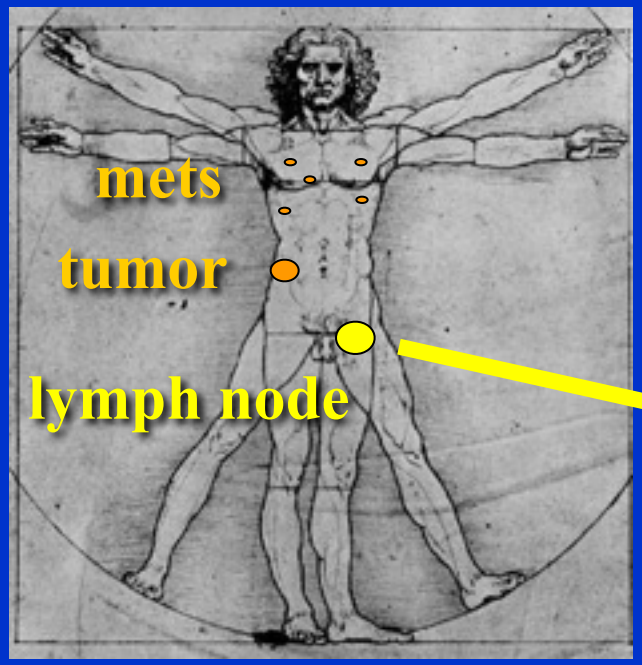


[5]J Natl Cancer Inst. 1987; 79(5): 1067-1075.

Immunotherapy for Cancer: 1990-2000

- optimal source of T cells?
  - tumor draining lymph nodes (TDLN)[6]
  - vaccine primed lymph nodes (VPLN)





α-CD3
IL-2

[6]Cancer Immunol Immunother. 1995; 83(1): 45-51.

Immunotherapy for Cancer: 2000-present

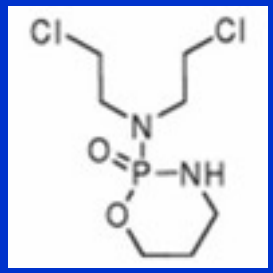
2 major areas –

Host – limits of immunodepletion prior to adoptive transfer

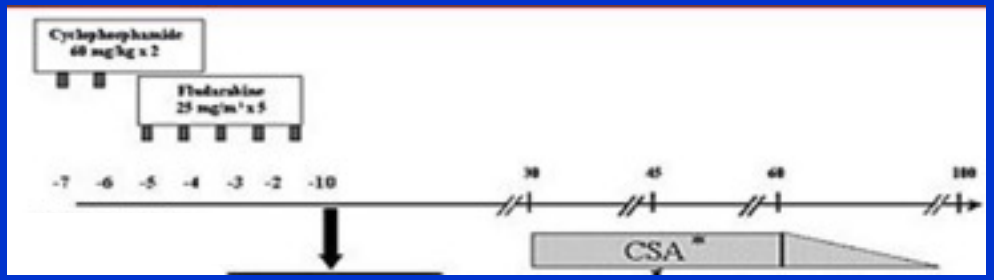
Effector – Genetically engineered T cells




Immunodepletion prior to adoptive transfer



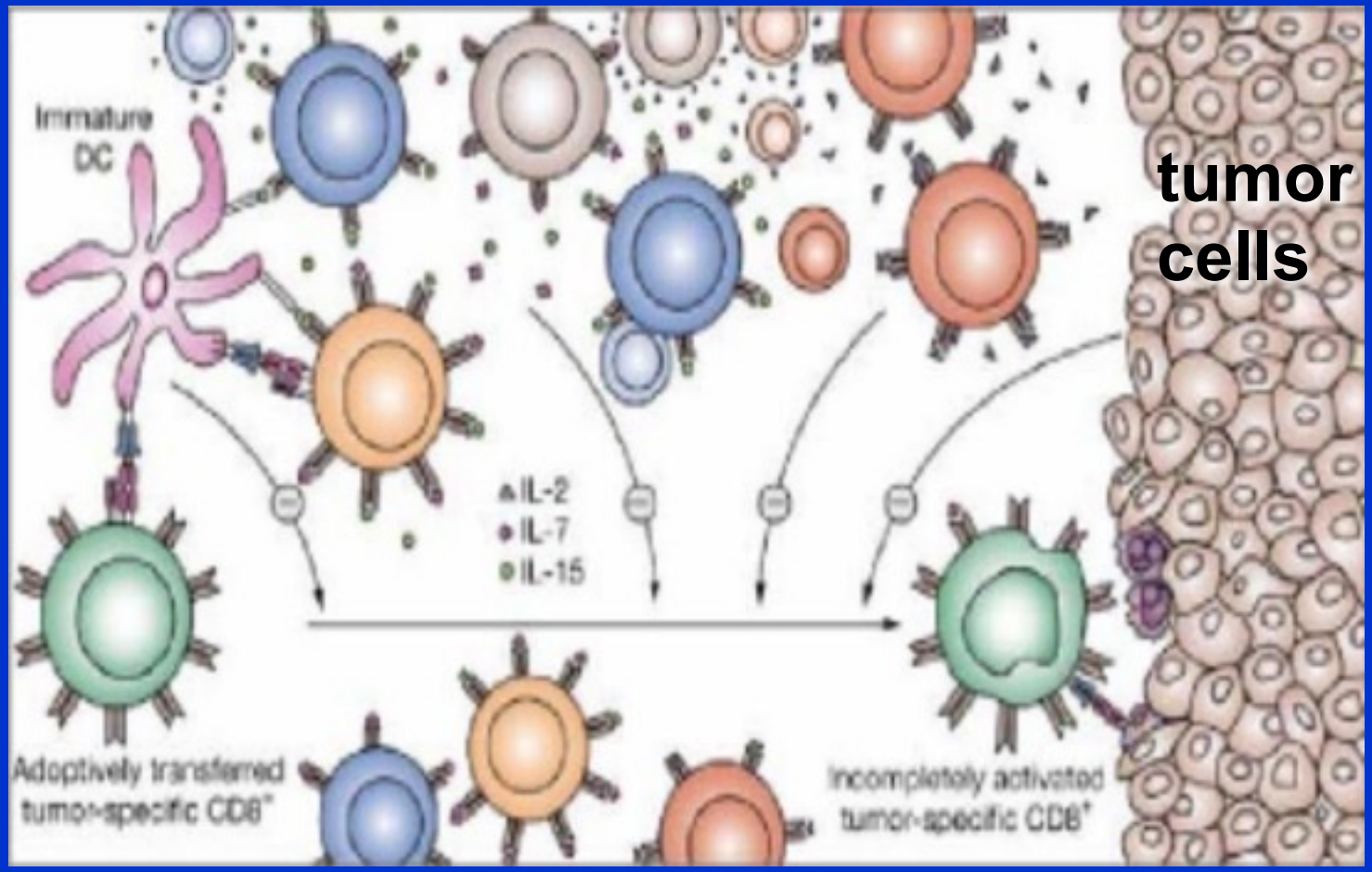
- eliminate competition for cytokines[7]
- eliminate suppressor cells (Tregs)
- generates maturation of dendritic cells
- increase in tumor antigen display



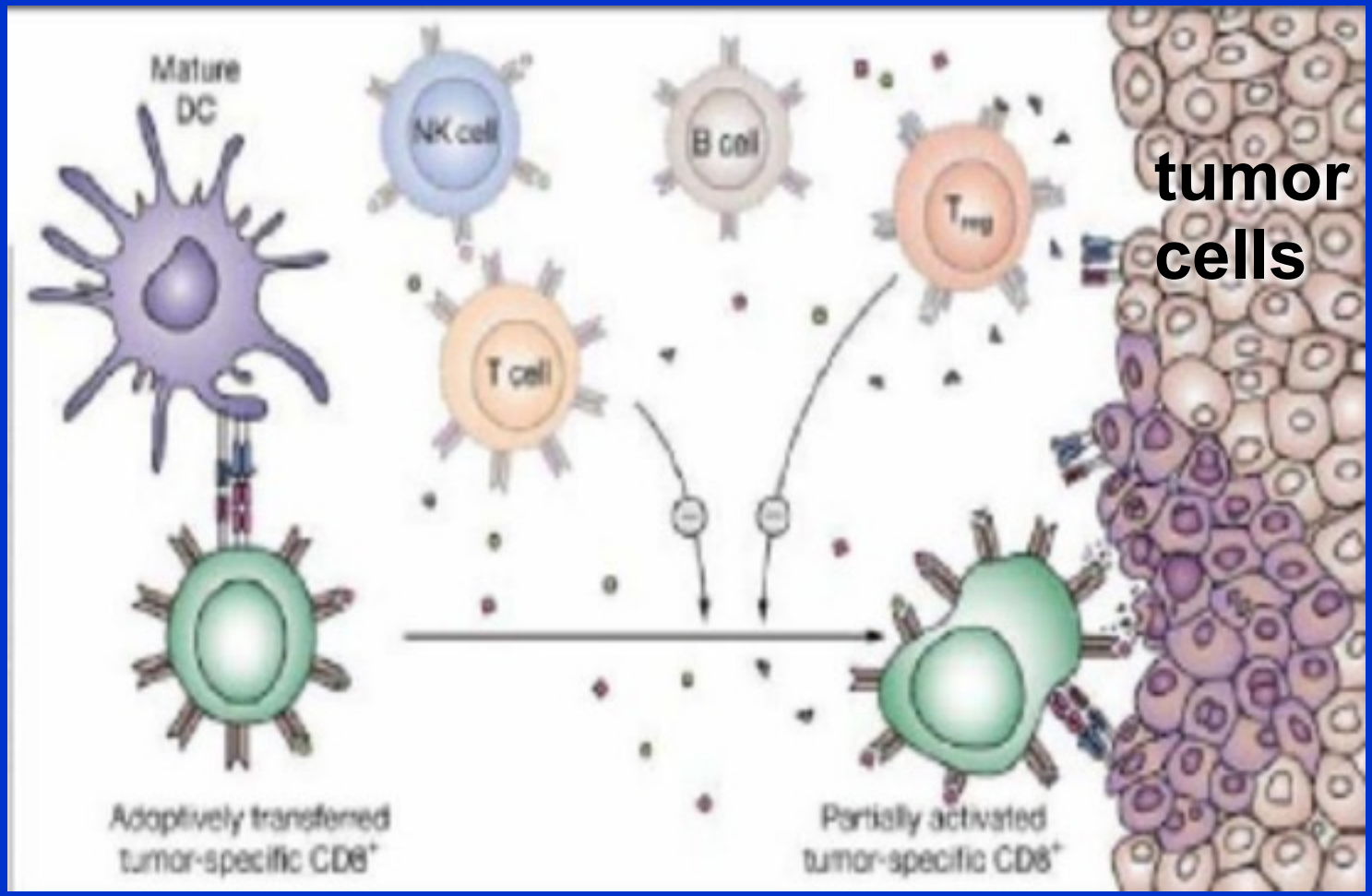
adoptive transfer

[7]J Immunother. 2010; 33(1): 1-7.

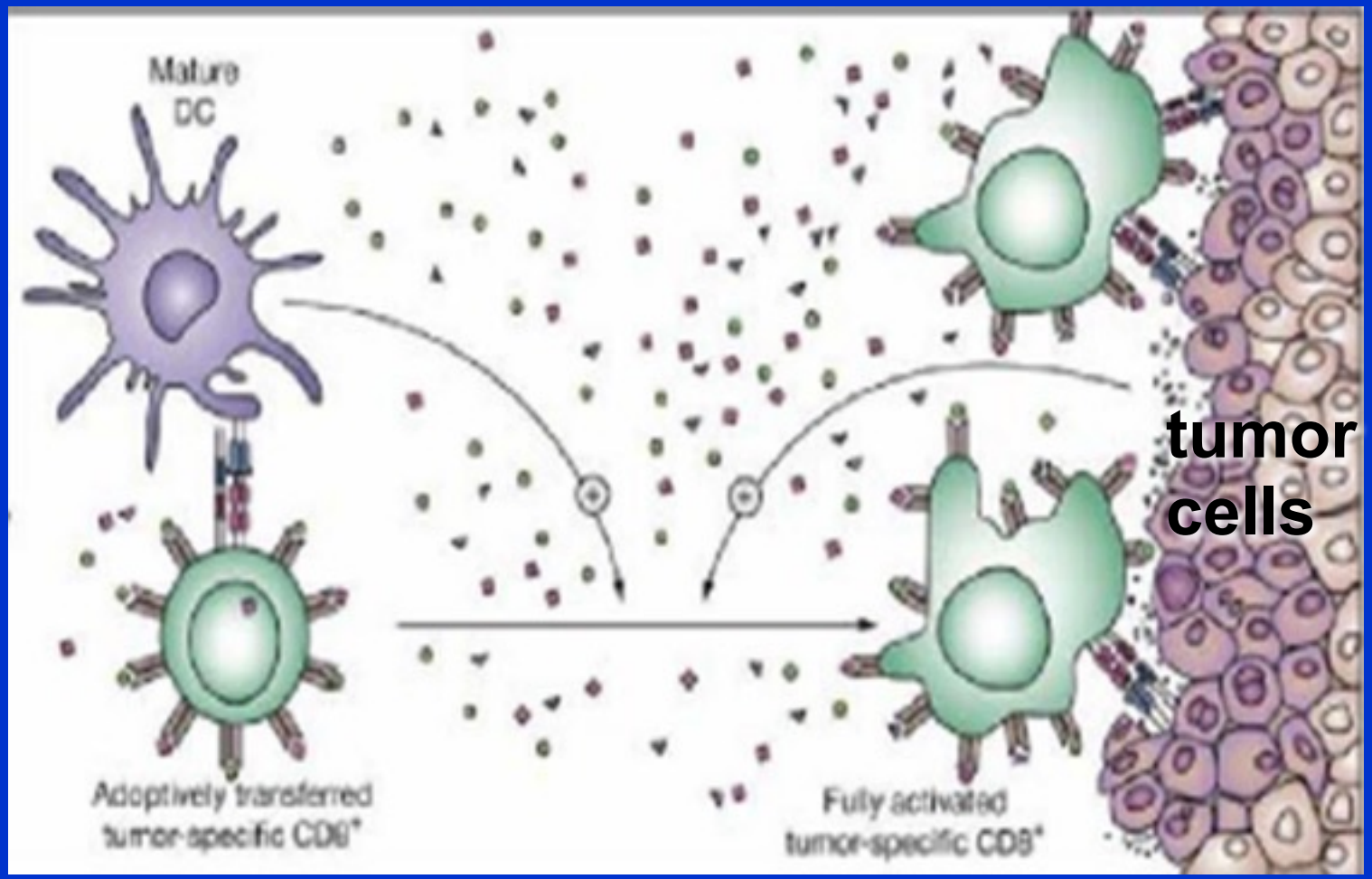
Immunocompetent host



Non-myeloalative preconditioning

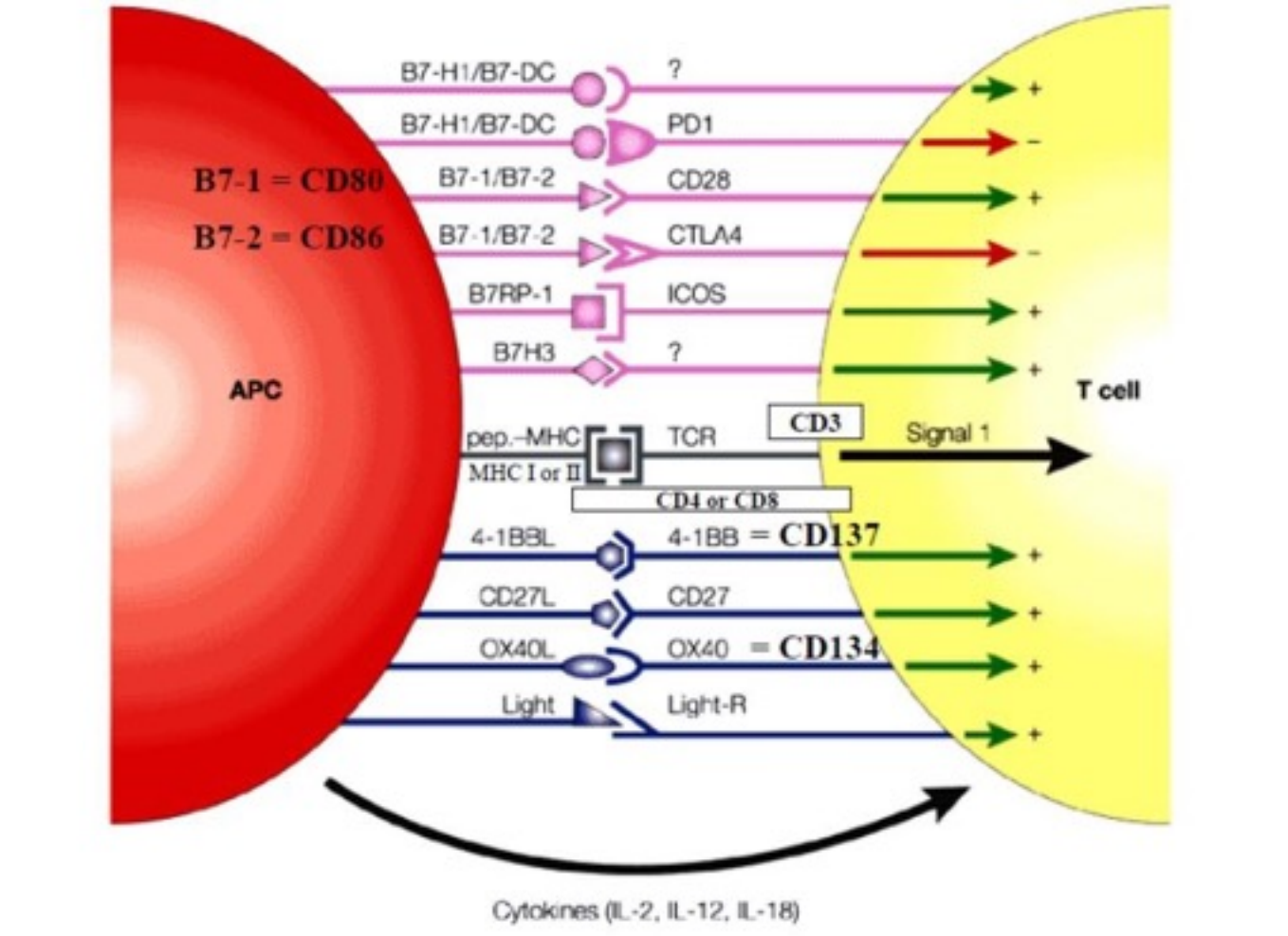


Myeloalative preconditioning

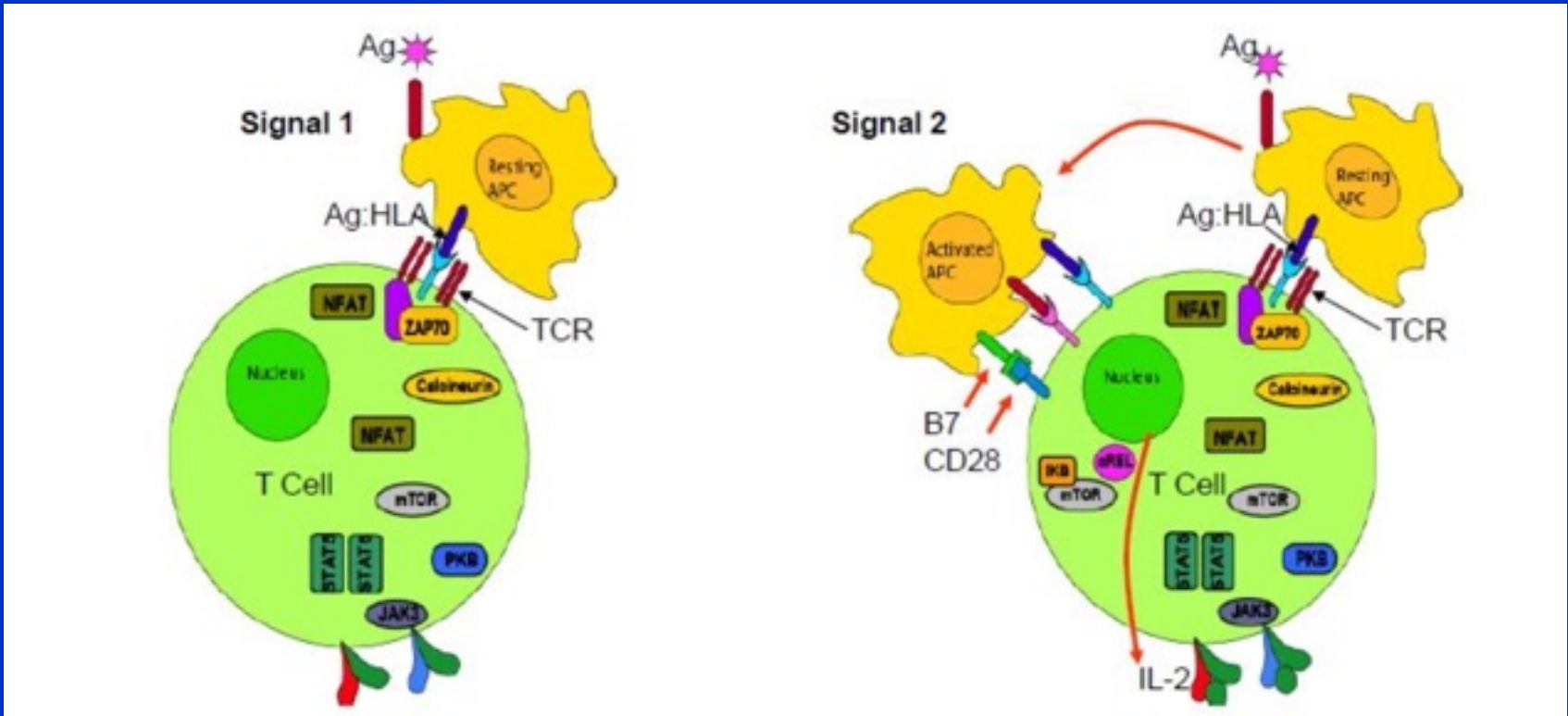


Genetically engineered T cells

- co-stimulation is complex and necessary



Antigenicity vs. Immunogenicity



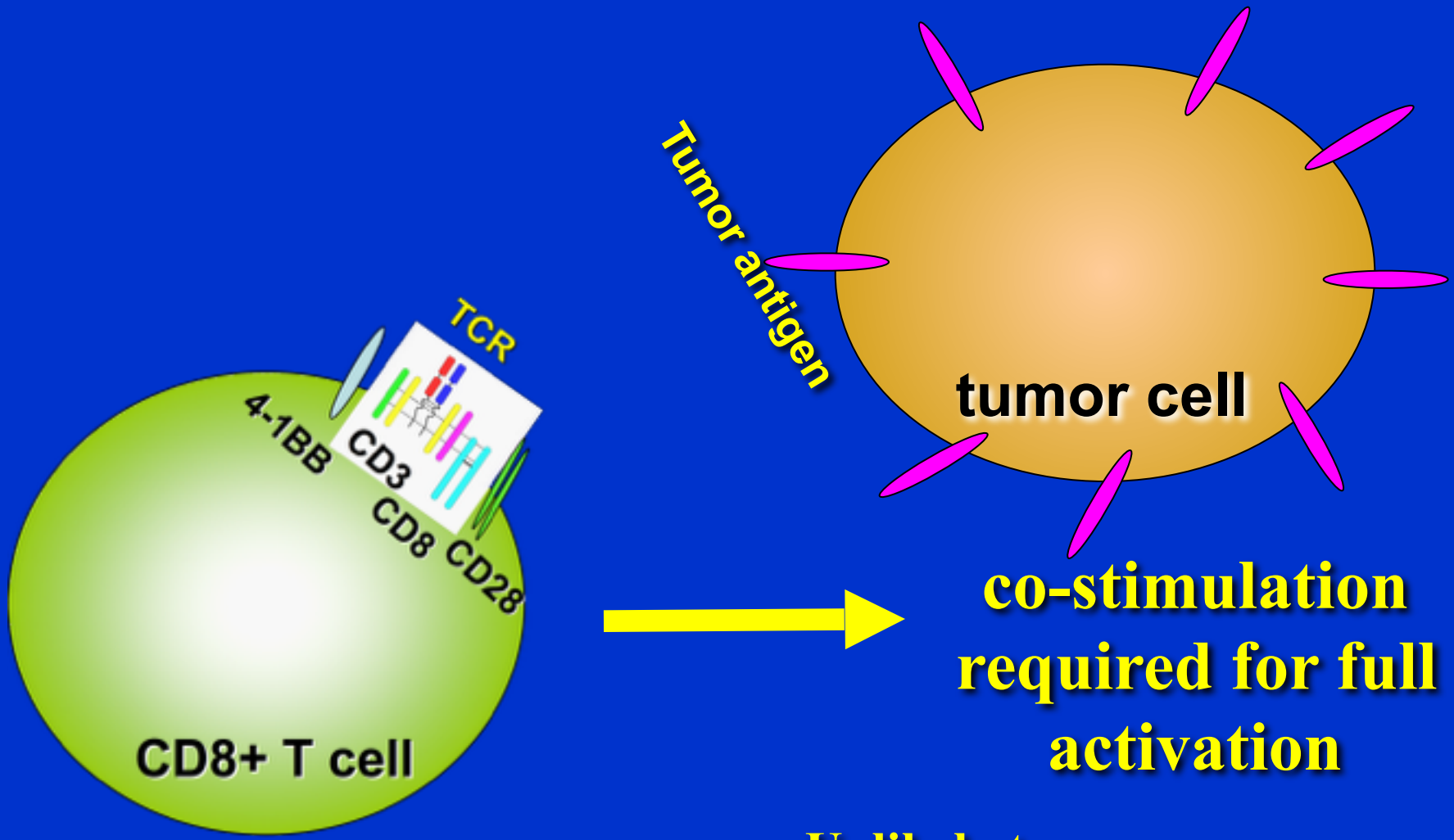
Antigen:APC T cell interaction, no costimulation

Result – T cell anergy, apoptosis, or Suppression (Treg)

Antigen:APC T cell interaction with costimulation

Result – T cell activation, clonal expansion effector functions

T cell activation and tumors

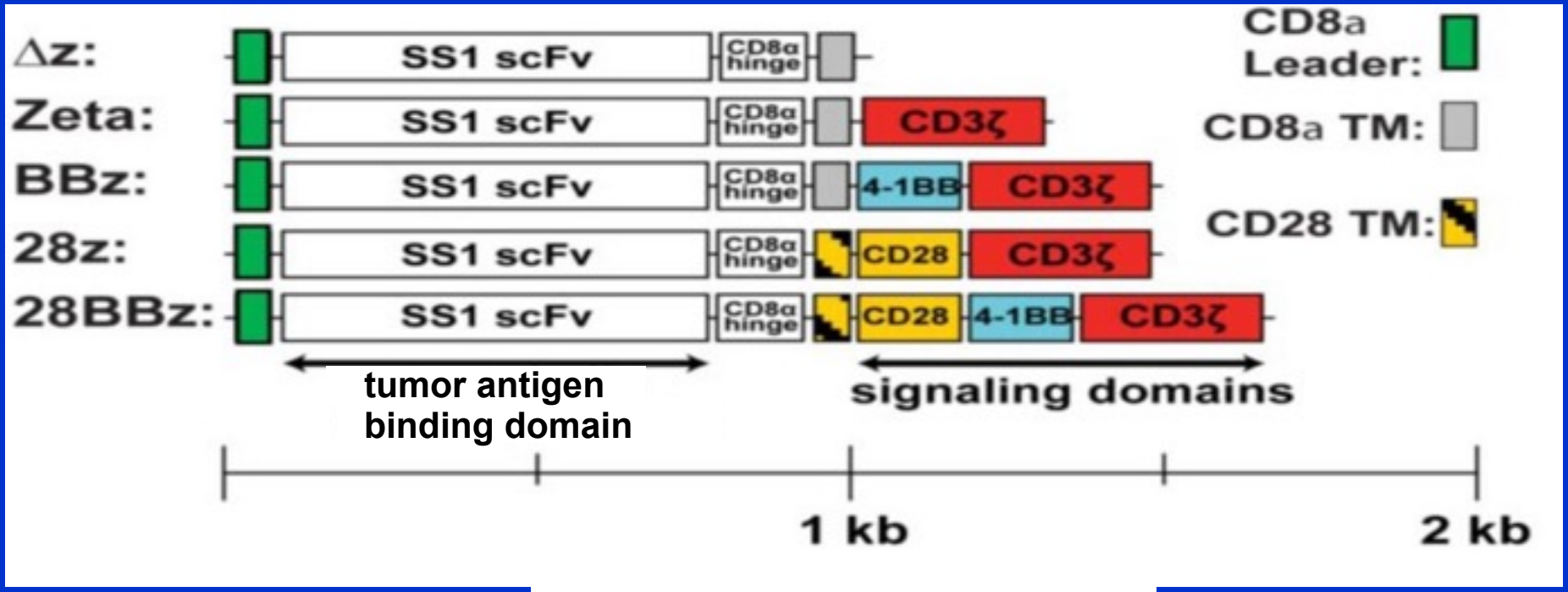


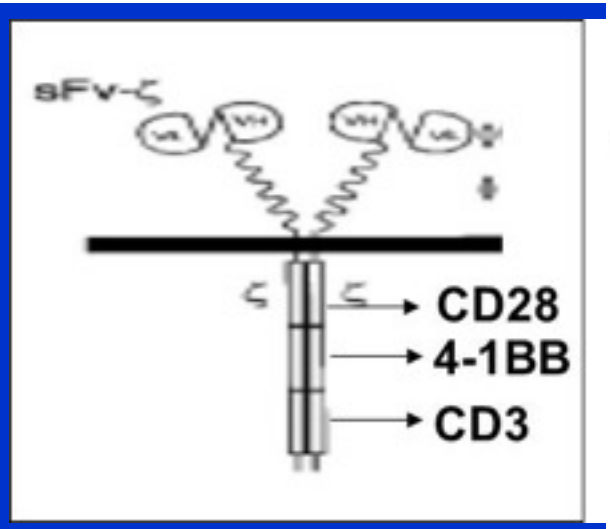
co-stimulation required for full activation

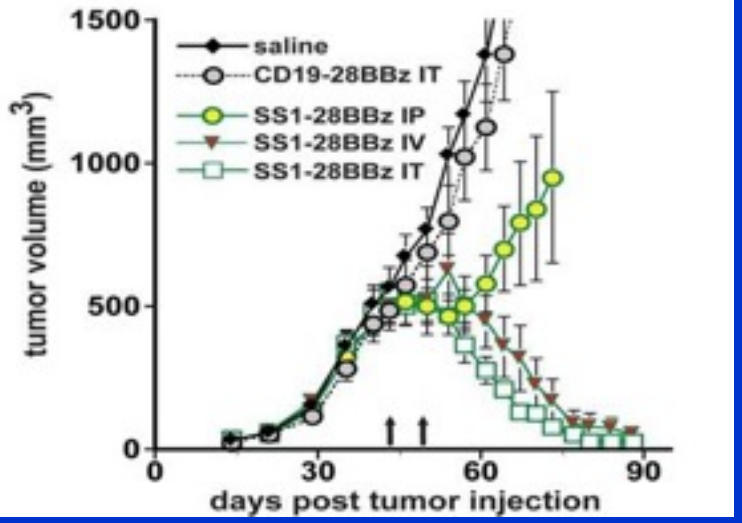
Unlikely to occur

- loss of MHC I
- lack of co-stimulatory molecules

Bypass activation requirements and TCR

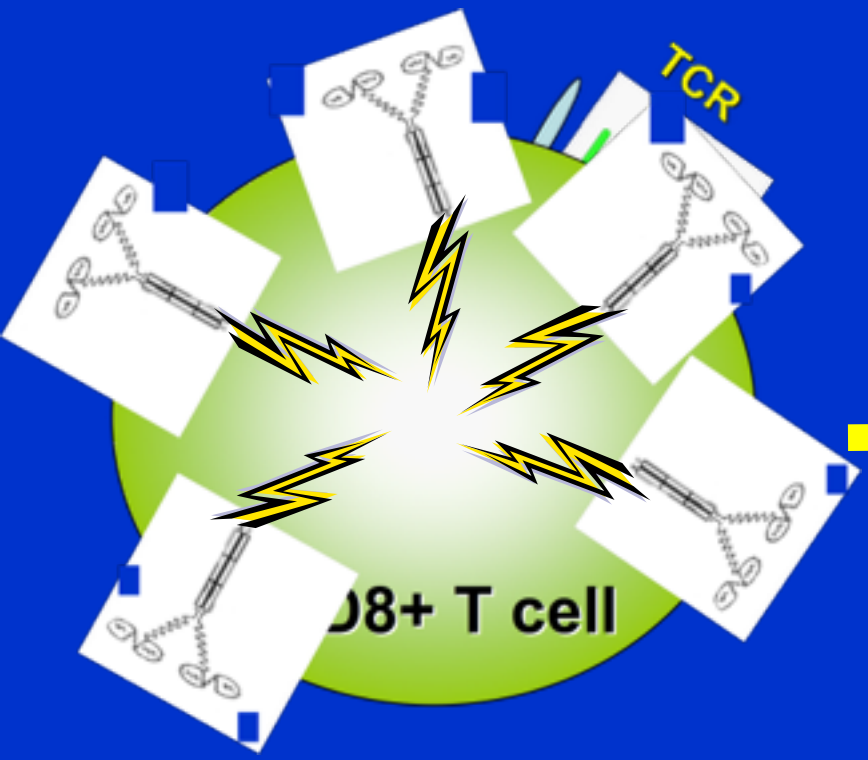


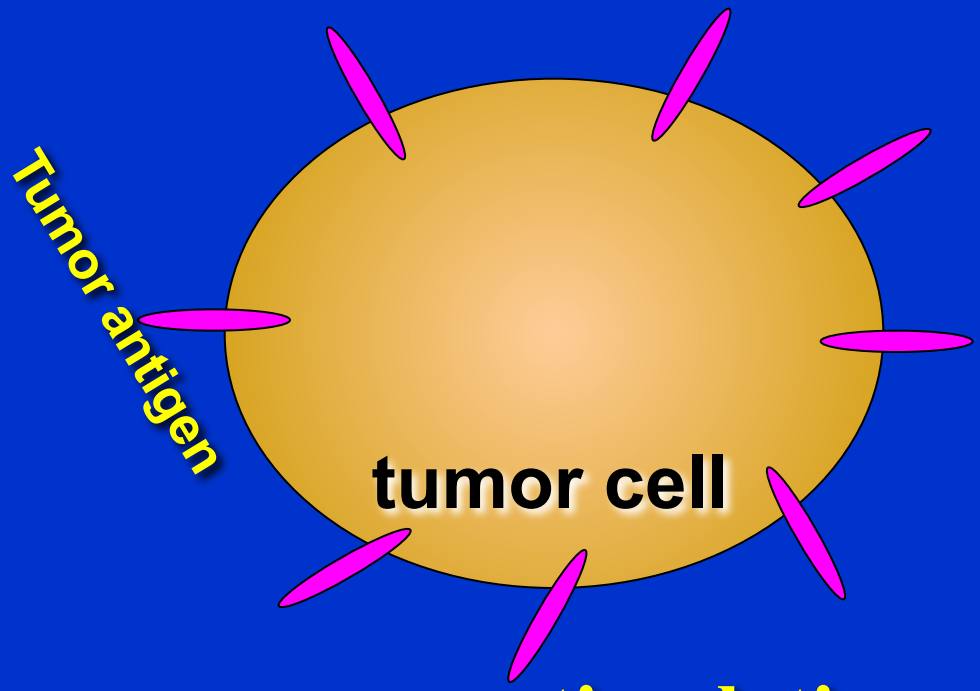




"CAR" *C*himeric *A*ntigen *R*eceptor

T cell activation and tumors





co-stimulation required for full activation

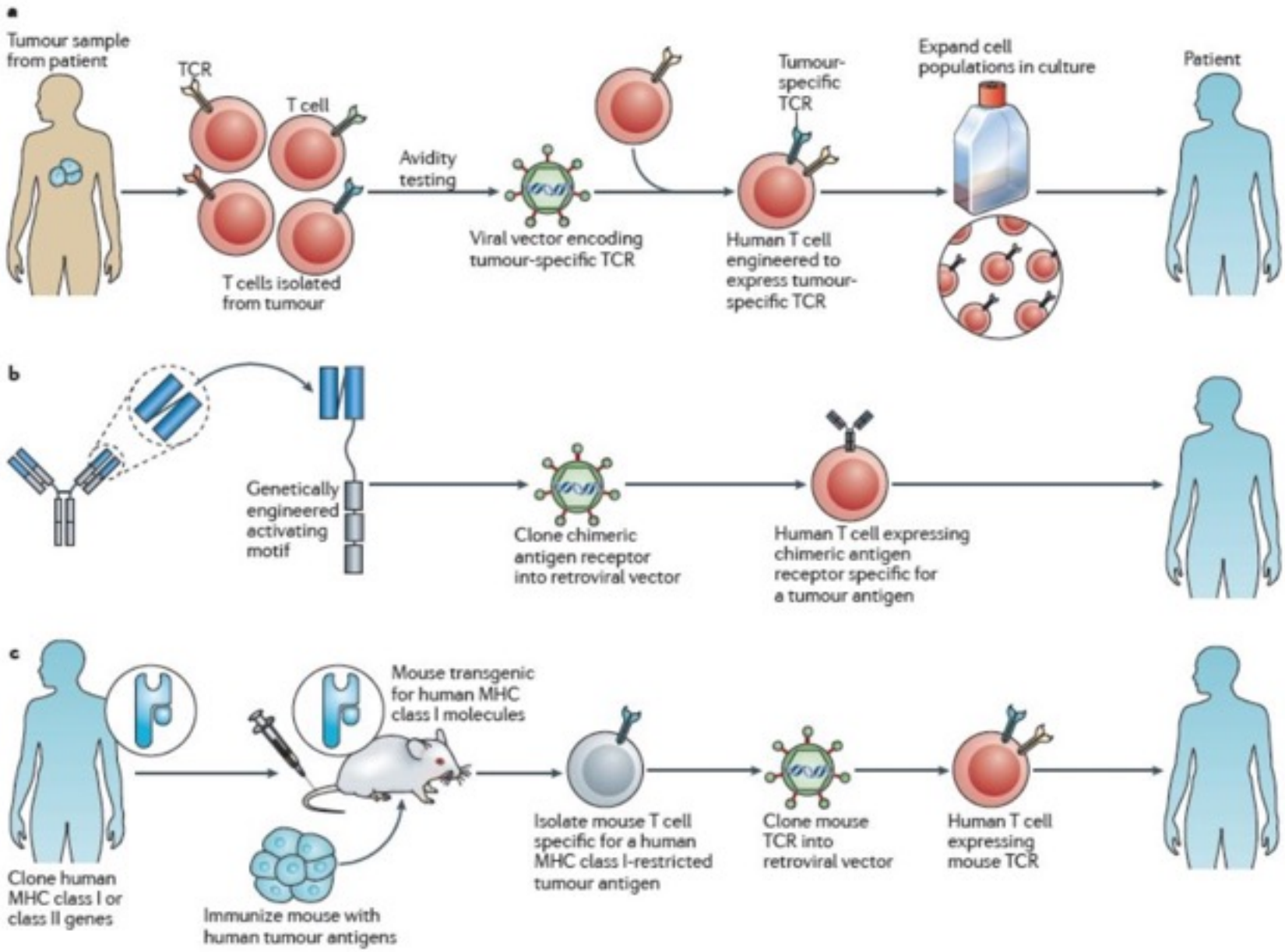
Unlikely to occur

- loss of efficient tumor cell killing
- lack of co-stimulatory molecules

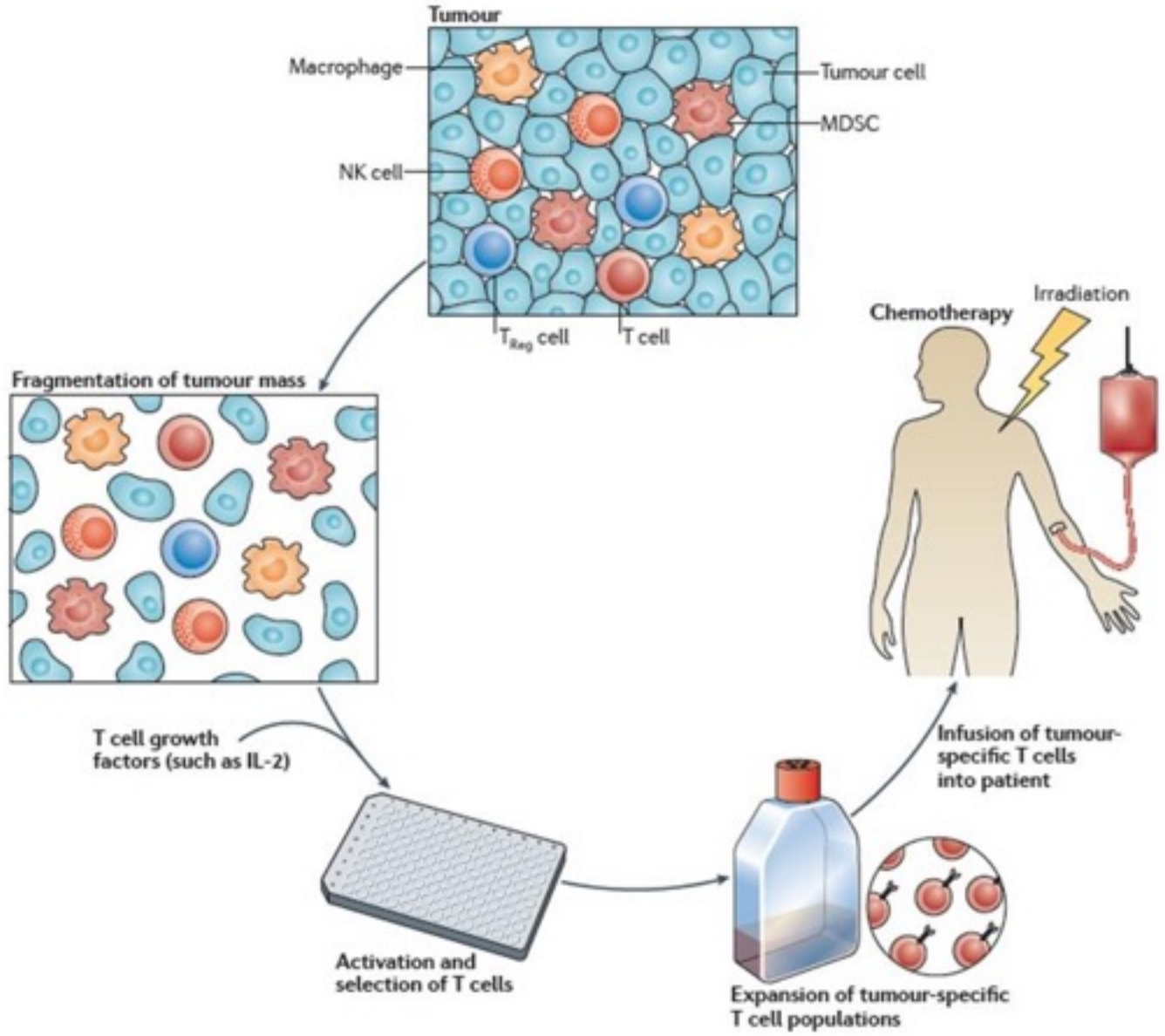
CAR activity

	Zeta only	28:z	41BB:z	28:41BB:z
kill	++	++	++	++
cytokine	+	+++	++	+++
Prolifera-tion	+	+++	+++	+++
In vivo survival	+	++	+++	+++

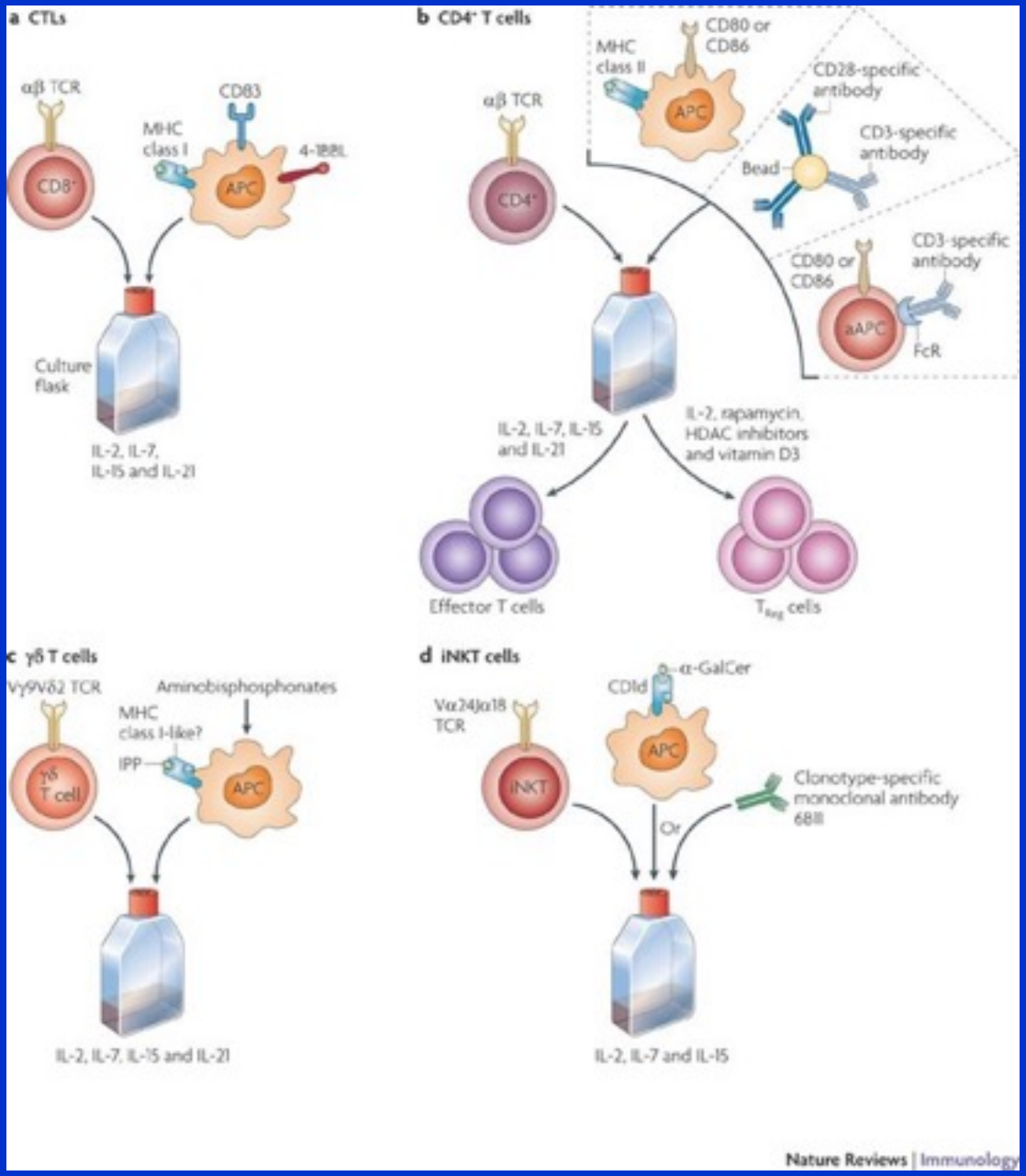
Other methods of engineering T cells



Adoptive Immunotherapy - Summary



Not limited to CD8 T cells



Response rates (not cure rate)

1994 – 34% with TIL

49% with TIL + immunodepletion

72% with TIL + immunodepletion + TBI

Why does adoptive immunotherapy fail?

- poor antigen display by tumor
- antigen loss
- hostile tumor microenvironment
- immune tolerance
- regulatory T cells
- insufficient numbers/persistence of cells
- inability to access tumors – limited lymphocyte trafficking

Antigen Loss

Antigen Loss

J Immunother. 2003 ; 26(5): 385–393.

Cell Transfer Therapy for Cancer: Lessons from Sequential Treatments of a Patient With Metastatic Melanoma

Steven A. Rosenberg[*], **James C. Yang**[*], **Paul F. Robbins**[*], **John R. Wunderlich**[*], **Patrick Hwu**[*], **Richard M. Sherry**[*], **Douglas J. Schwartzentruber**[*], **Suzanne L. Topalian**[*], **Nicholas P. Restifo**[*], **Armando Filie**[†], **Richard Chang**[‡], and **Mark E. Dudley**[*]

**Center for Cancer Research, Surgery Branch, National Cancer Institute, National Institute of Health, Bethesda, Maryland*

†Center for Cancer Research, Laboratory of Pathology, National Cancer Institute, National Institute of Health, Bethesda, Maryland

‡Diagnostic Radiology Department, Clinical Center, National Cancer Institute, National Institute of Health, Bethesda, Maryland

A unique case study

- 1997 – 27 year old female with melanoma of the eyelid

- excised, but recurred in 1999

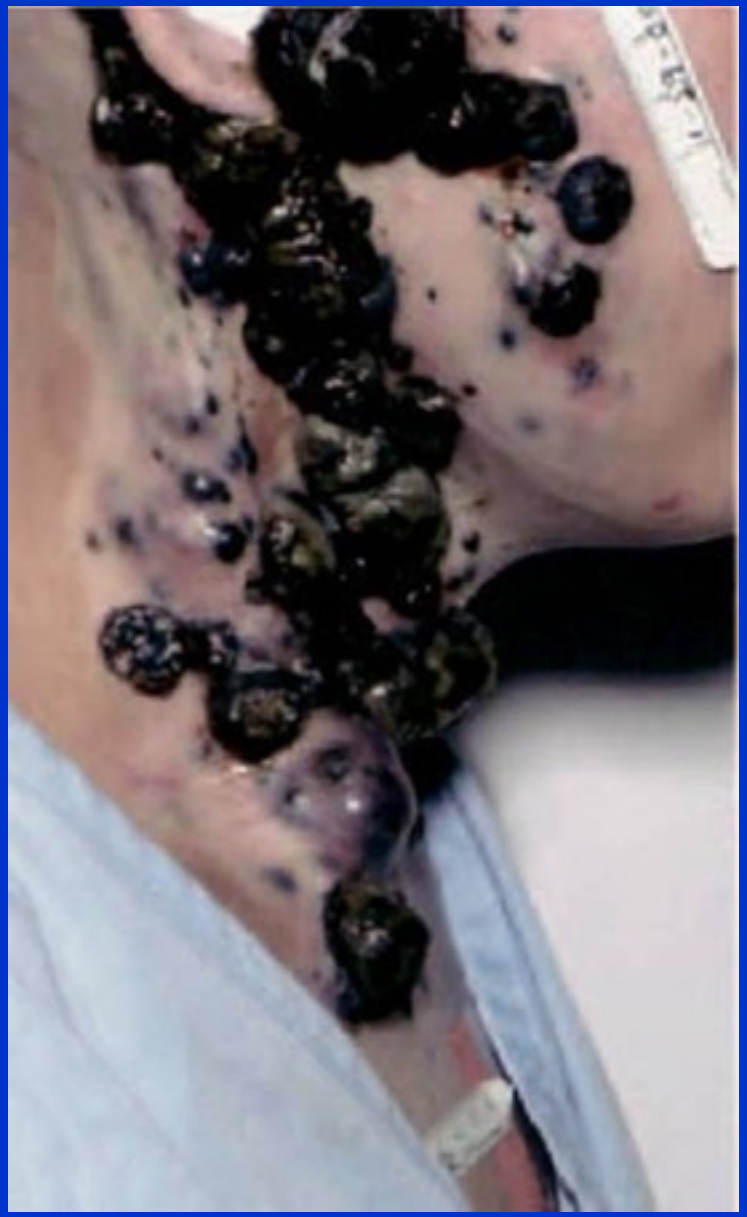
- treated with superficial parotidectomy, cervical lymph node dissection, 60 Gy to her face and neck, and bio-chemotherapy – chemo + IL-2 + alpha-interferon

- disease progressed referred to NCI in 2000

Treated with peptide vaccine and IL-2

gp 100 antigen

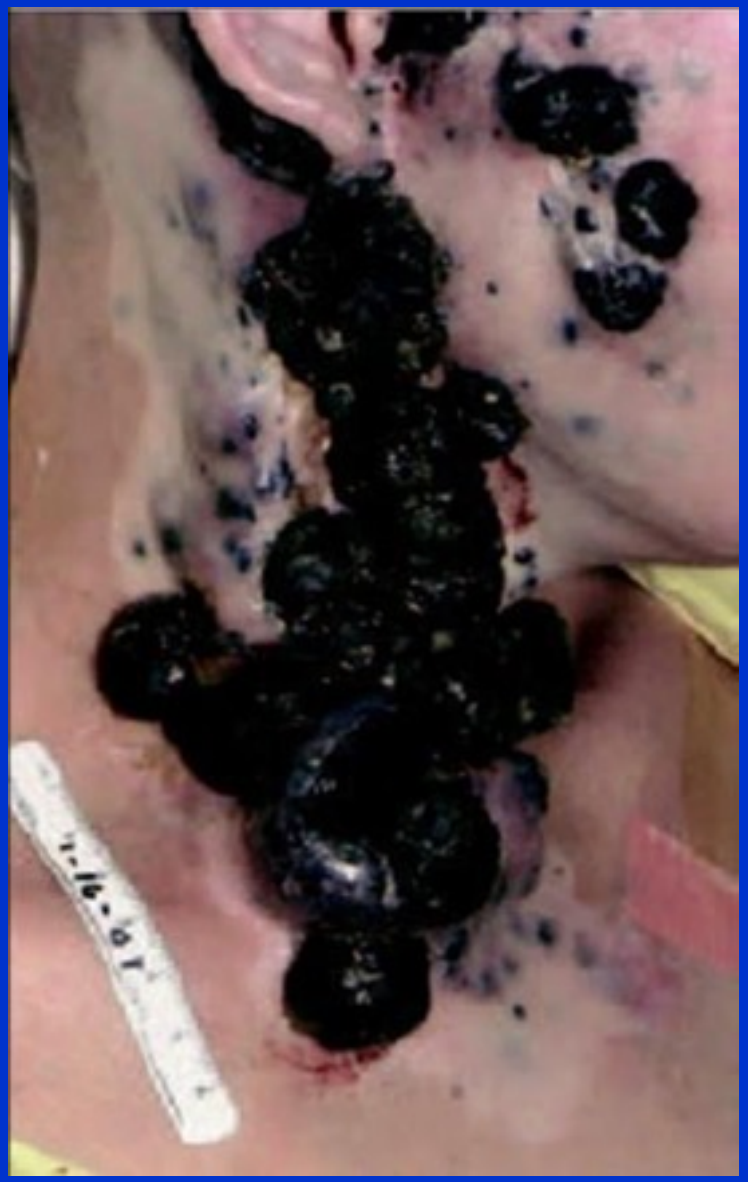
November 2000



Adoptive transfer x5

Treatment #1 –
<u>autologous lymphocytes</u>

- 1 source – PBL
- reactive gp100
- immunodepletion prior
- $1x10^{10}$ cells injected i.v.
- minimal response

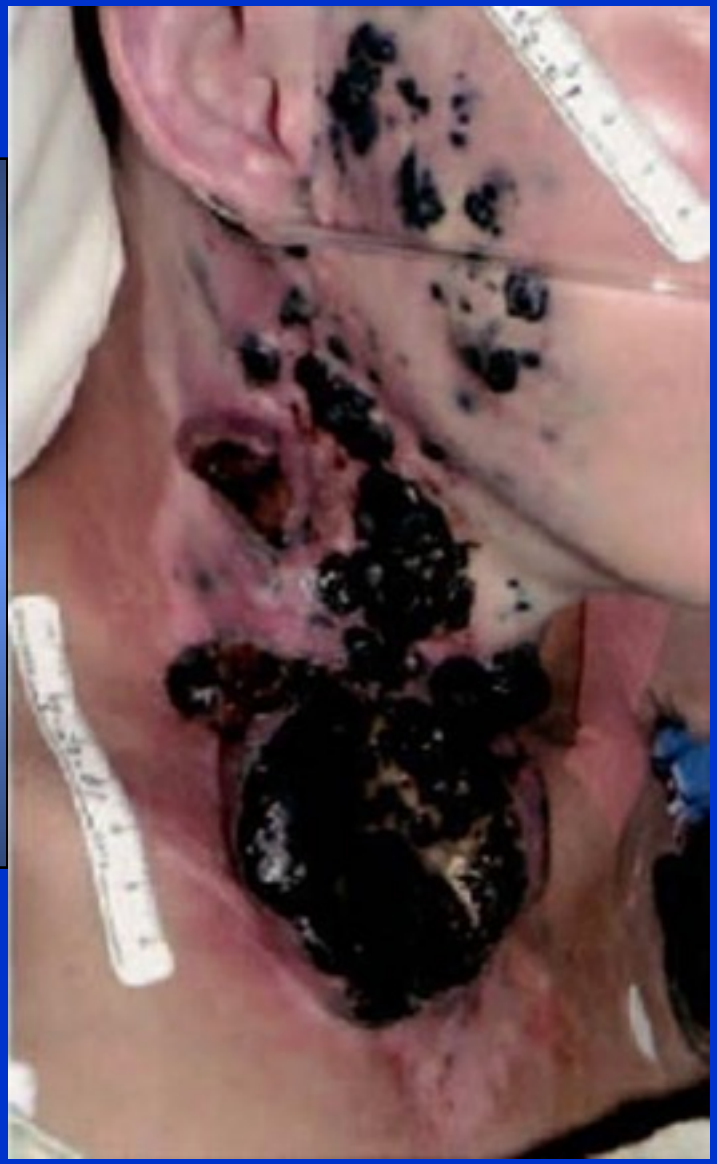


January 2001

Lymphodepletion improves response

Treatment #2, #3 –
<u>autologous lymphocytes</u>

- 2 sources – PBL + TIL
- reactive gp100, MART-1
- immunodepletion prior to #3
- ~$4x10^{10}$ cells injected i.v.
- improved response



March 2001

Lymphocyte trafficking is important

Treatment #4 –
<u>autologous lymphocytes</u>

- 2 sources – PBL + TIL
- reactive gp100, MART-1
- immunodepletion prior
- ~$4x10^{10}$ cells injected i.a.
- much improved response

May 2001

Antigen loss is real and leads to tumor escape

Treatment #5 –

- Patient refused surgery for growing nodules of neck

- died of progressive melanoma December 2001

- no response
- tumor biopsy revealed a single point mutation and loss of HLA-A2 antigens

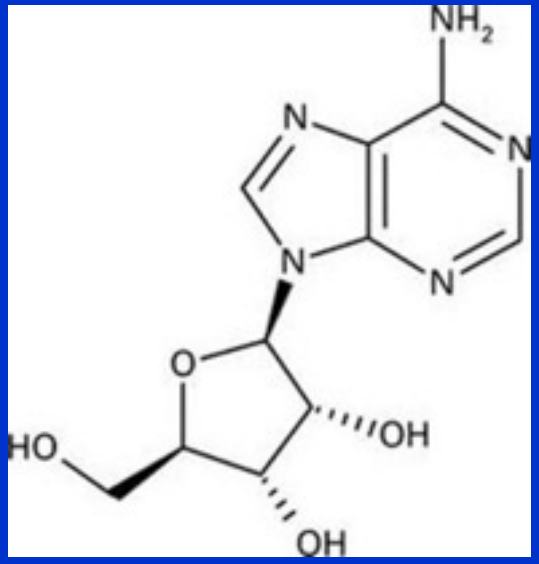
Hostile Tumor Microenvironment

- Adenosine
- Indolemine 2,3 dioxygenase (IDO)
- hypoxic
- acidotic
- lack of co-stimulation/cytokines

Adenosine

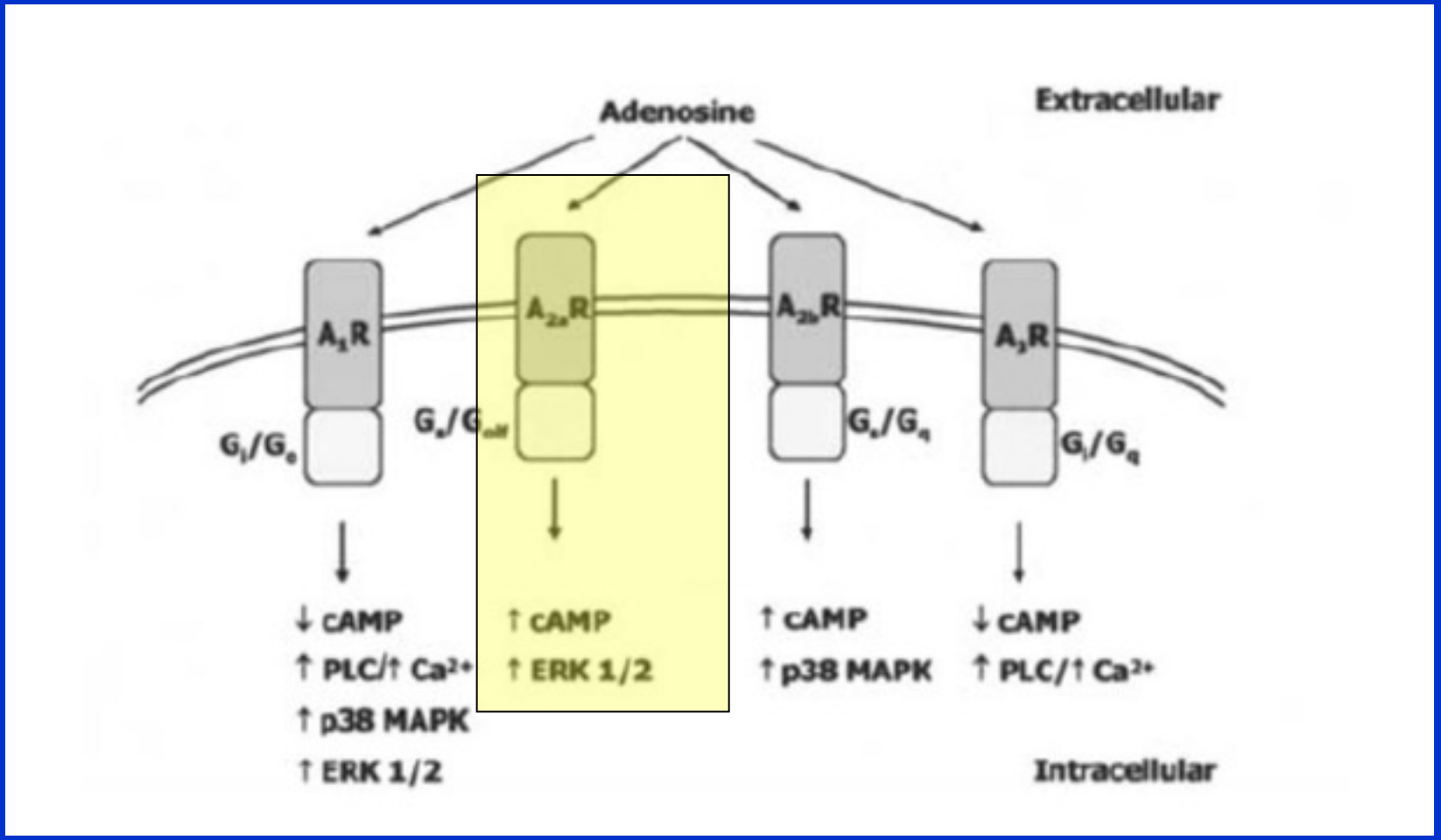
Adenosine

- damaged tissues – nucleotidases
- ATP/AMP converted to adenosine
- A1, A2A, A2B, and A3 receptors
- inhibits activation and expansion of T cells[8]
- "Hellstrom paradox"
- normally a protective mechanism



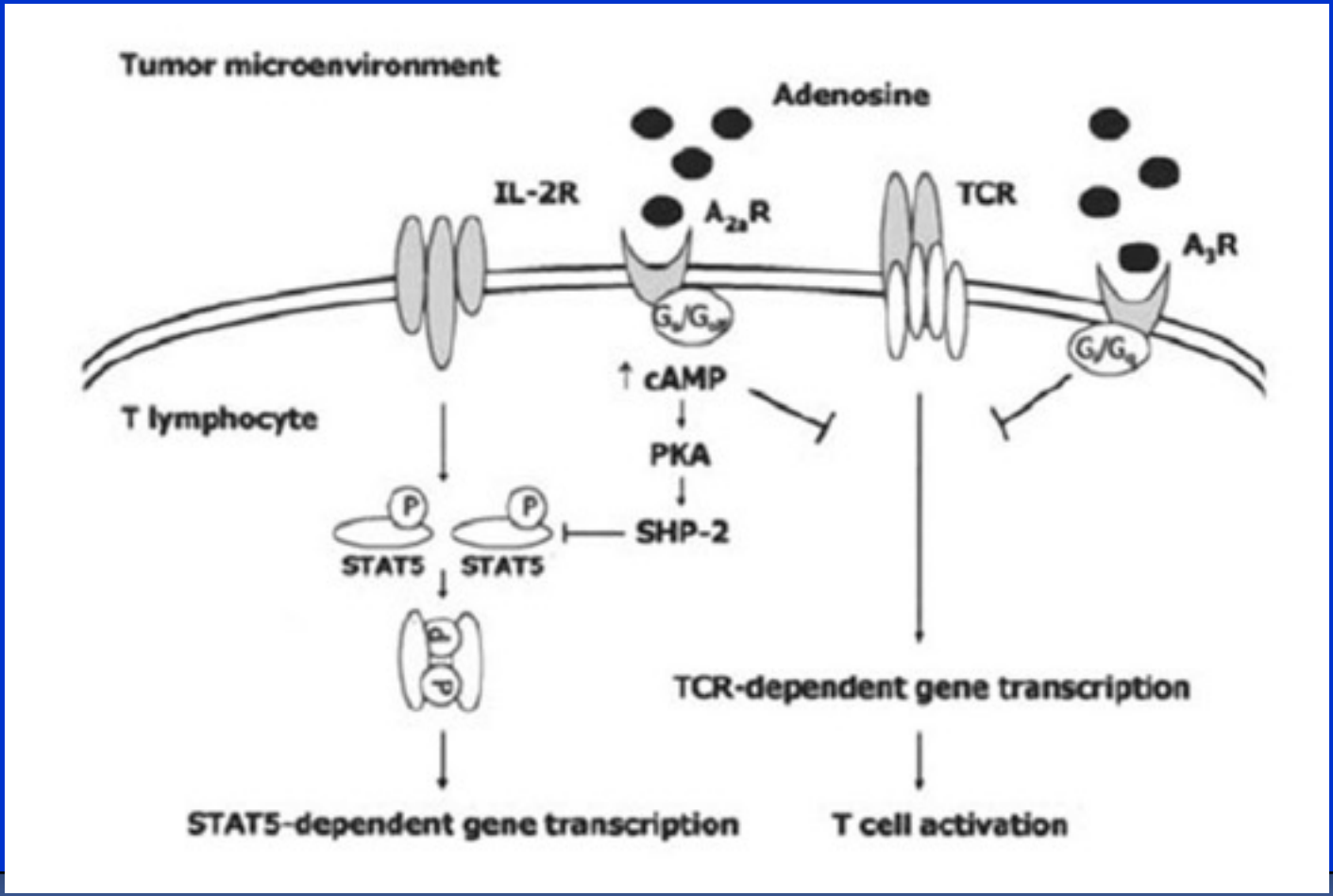
[8]PNAS 2006; 103(35): 13132-13137.

Adenosine



[8]Int J Oncol 2008; 32(3): 527-35.

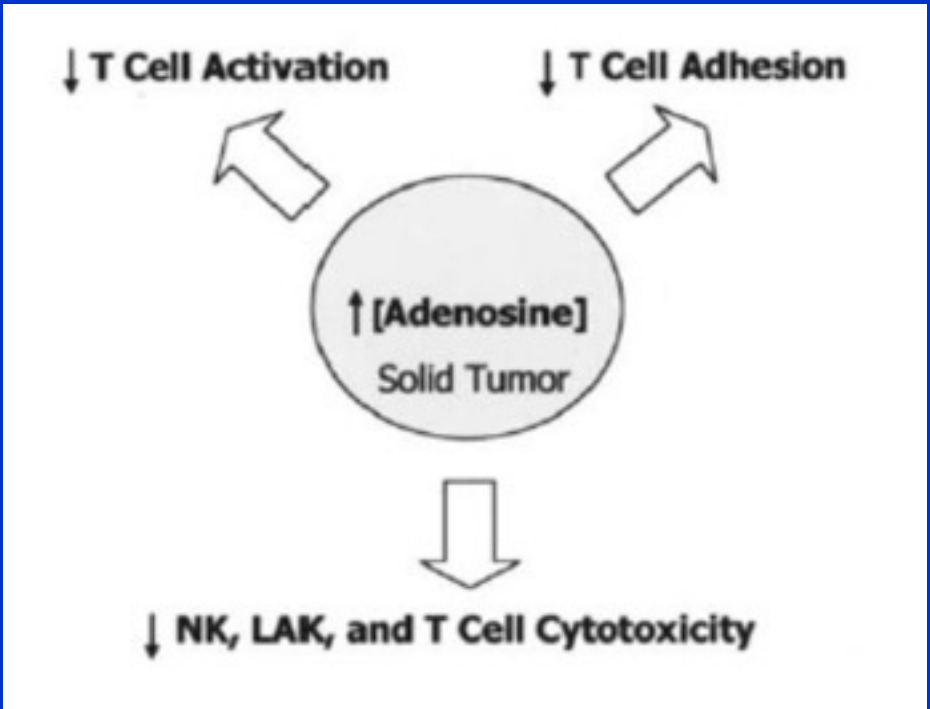
Adenosine



- inhibits both activation and expansion
- SHP-2 – tyrosine phosphatase
- inhibits P56 and ZAP-70

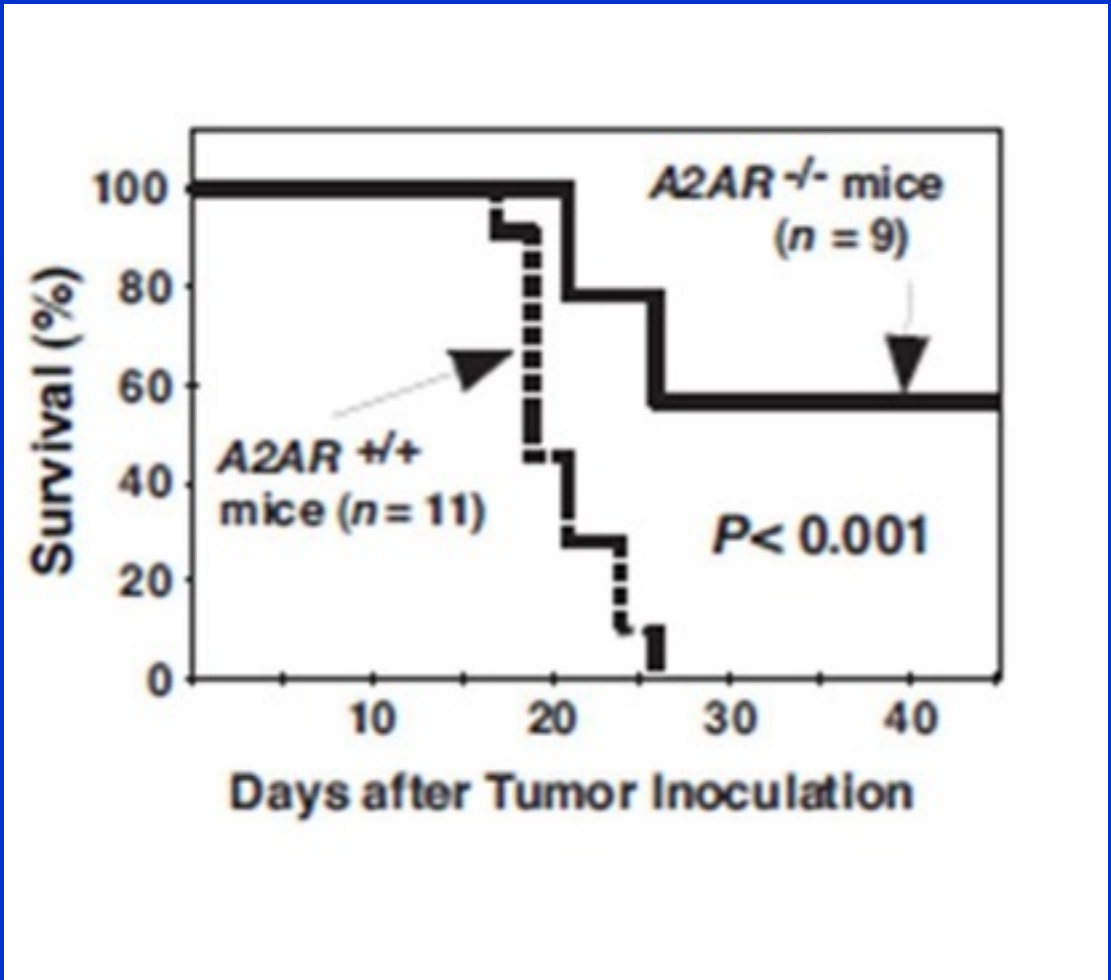
[8]Int J Oncol 2008; 32(3): 527-35.

Adenosine



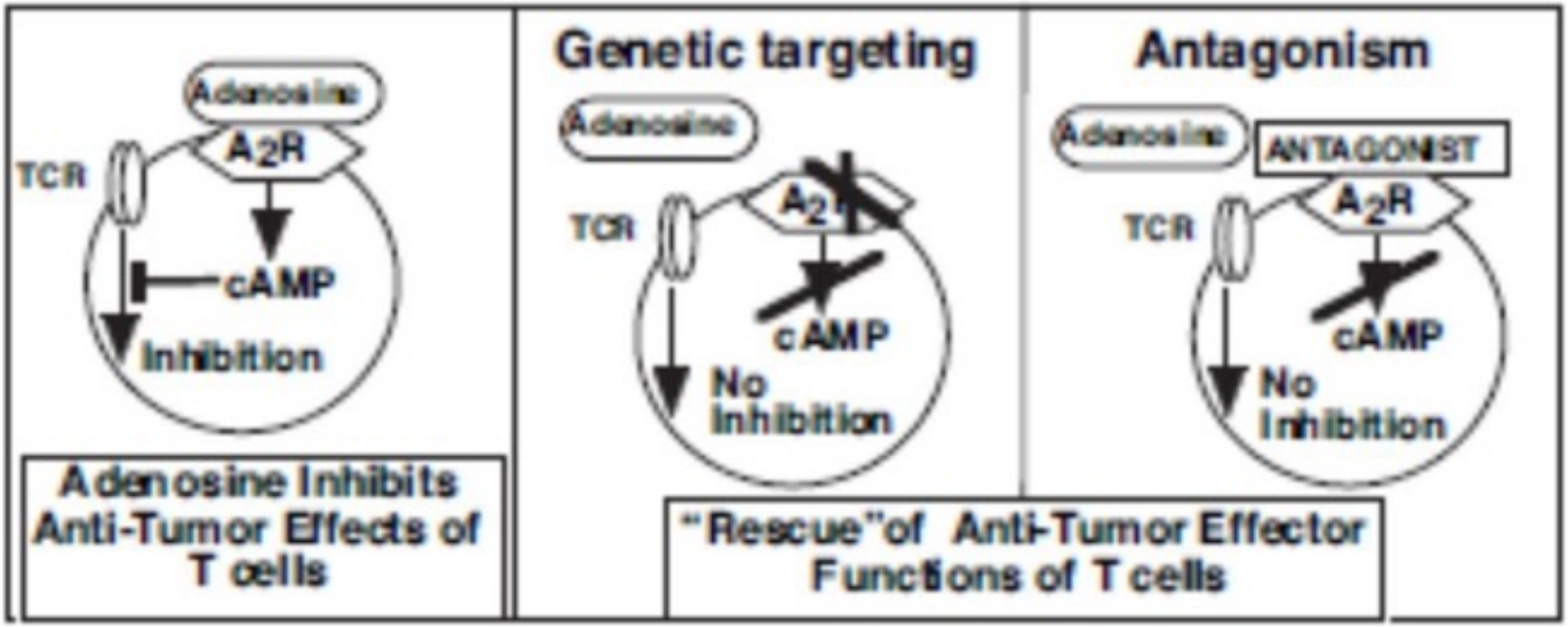
- Tregs – CD39 and CD73 – sequentially catalyze to generate adenosine

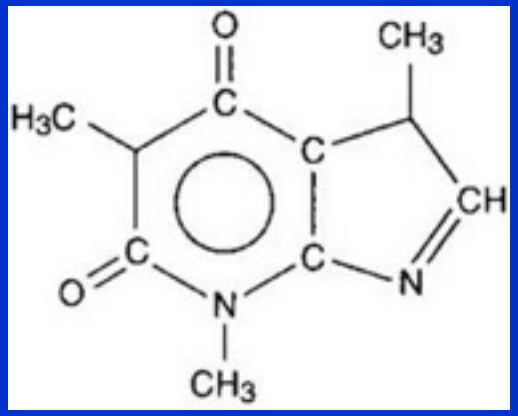
Adenosine



Effects of adenosine receptor-mediated, so . . . .

Adenosine





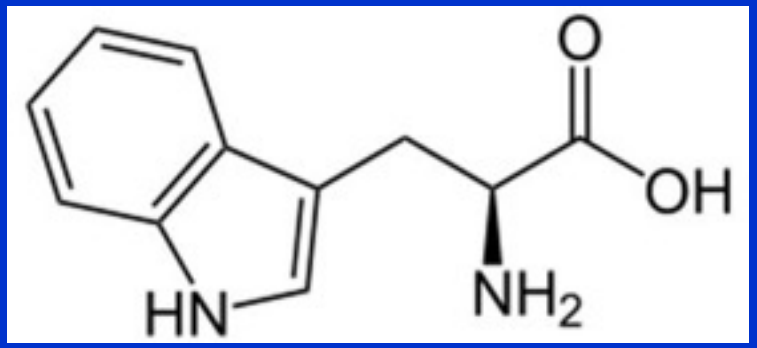
- physiologic doses

[8]PNAS 2006; 103(35): 13132-13137.

Indolemine 2,3 dioxygenase (IDO)

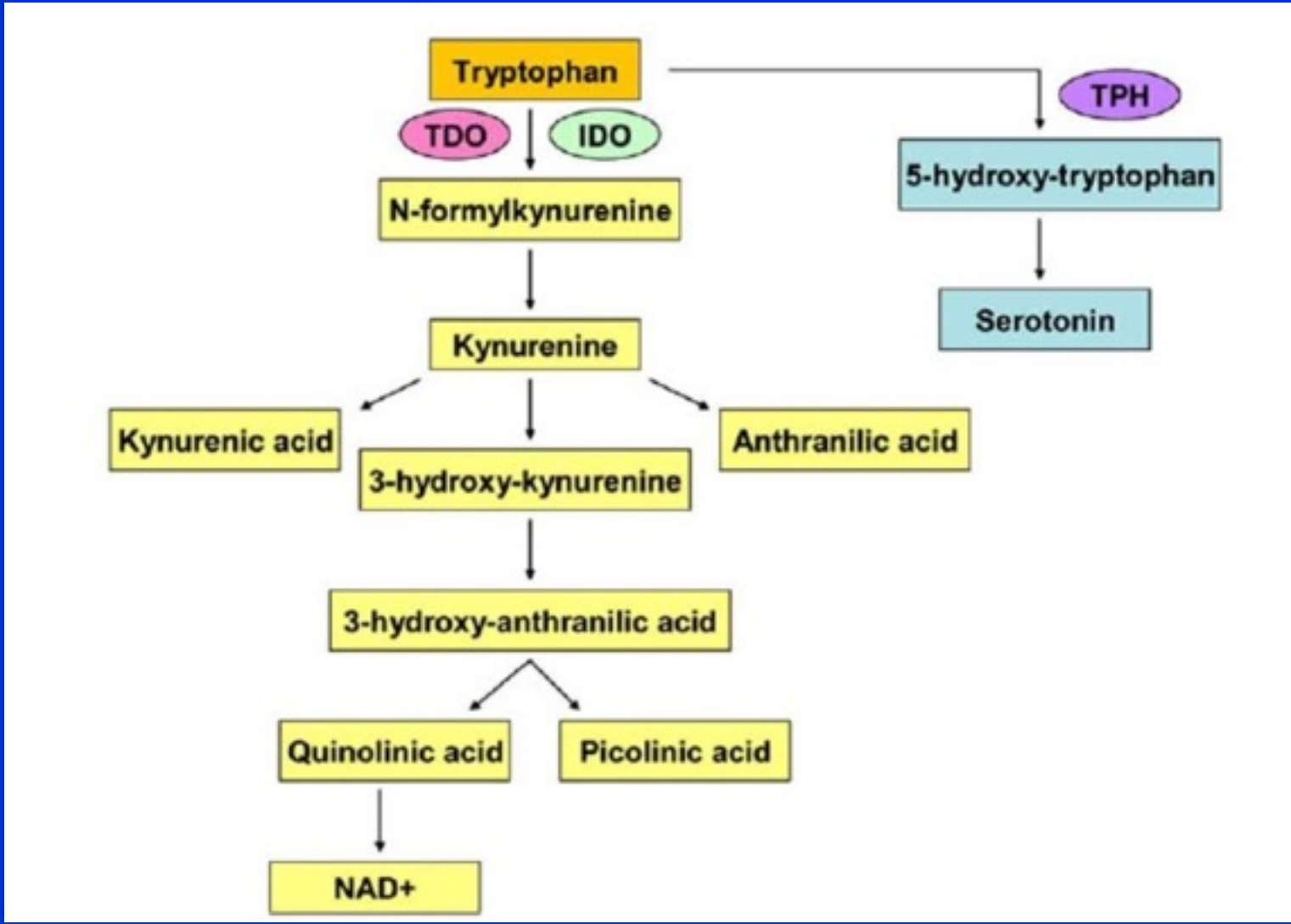
Indolemine 2,3 dioxygenase (IDO)

- metabolizes tryptophan
- responsible for tumor tolerance[9]
  - primary tumor site
  - draining lymph node
- normally – upregulated in APC in response to INF-γ (negative feedback loop)
- major mechanism of Treg

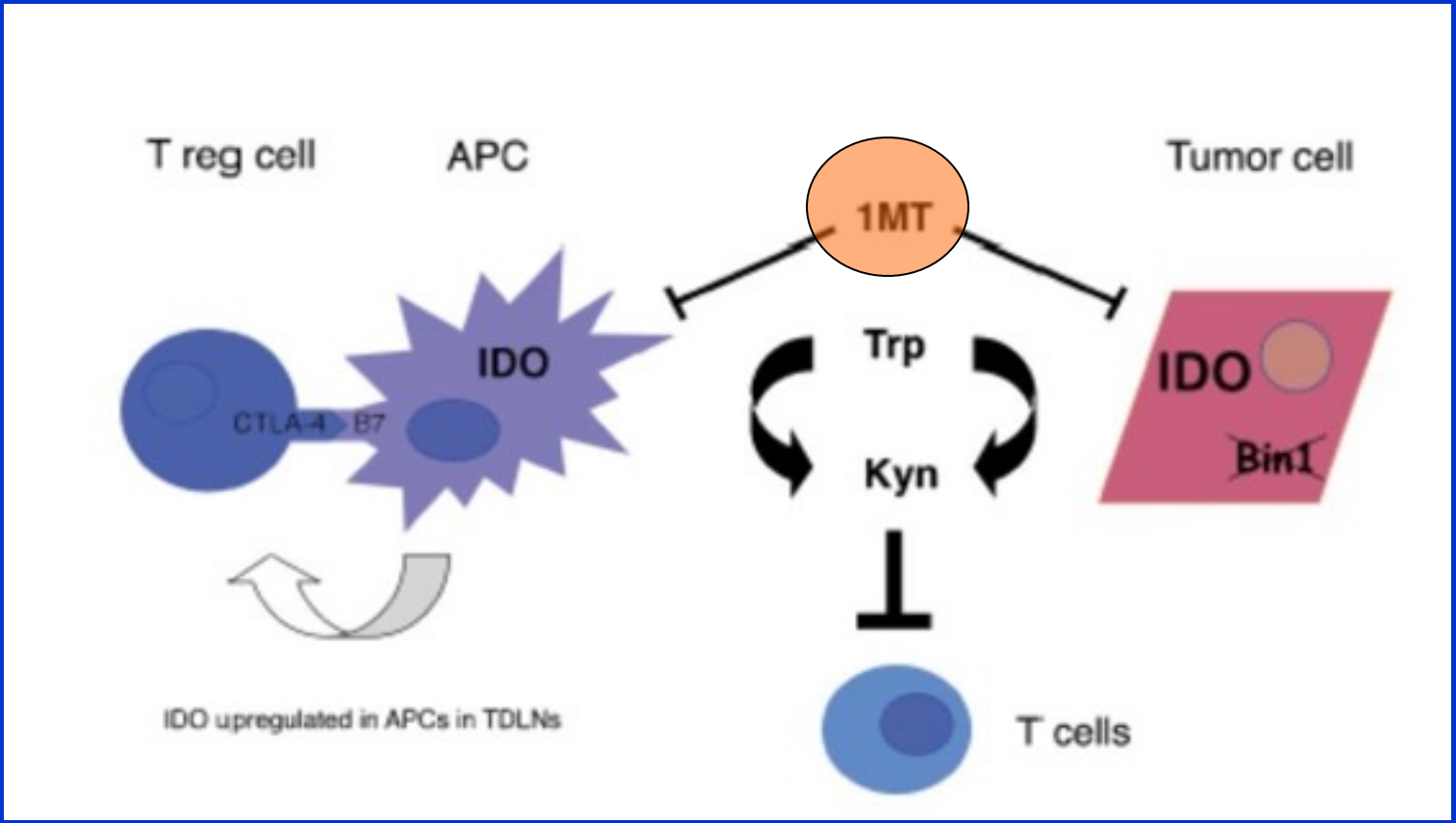


[9]Immun Rev 2008; 222: 206-221.

Tryptophan catabolic pathways



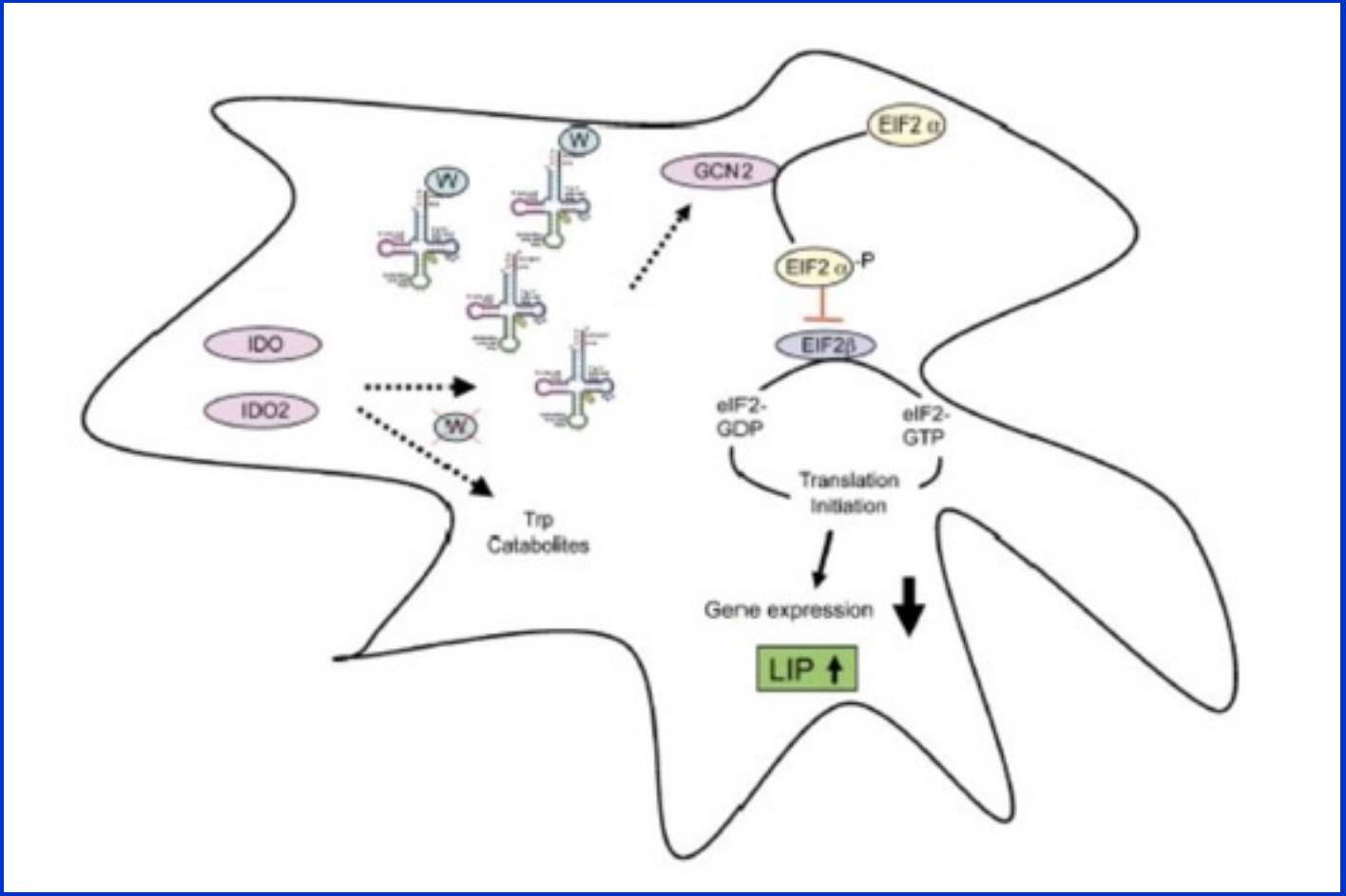
Indolemine 2,3 dioxygenase (IDO)



- IDO dysregulated in tumor due to Bin1 loss
- 1MT inhibitor of IDO in clinical trials

[9]Immun Rev 2008; 222: 206-221.

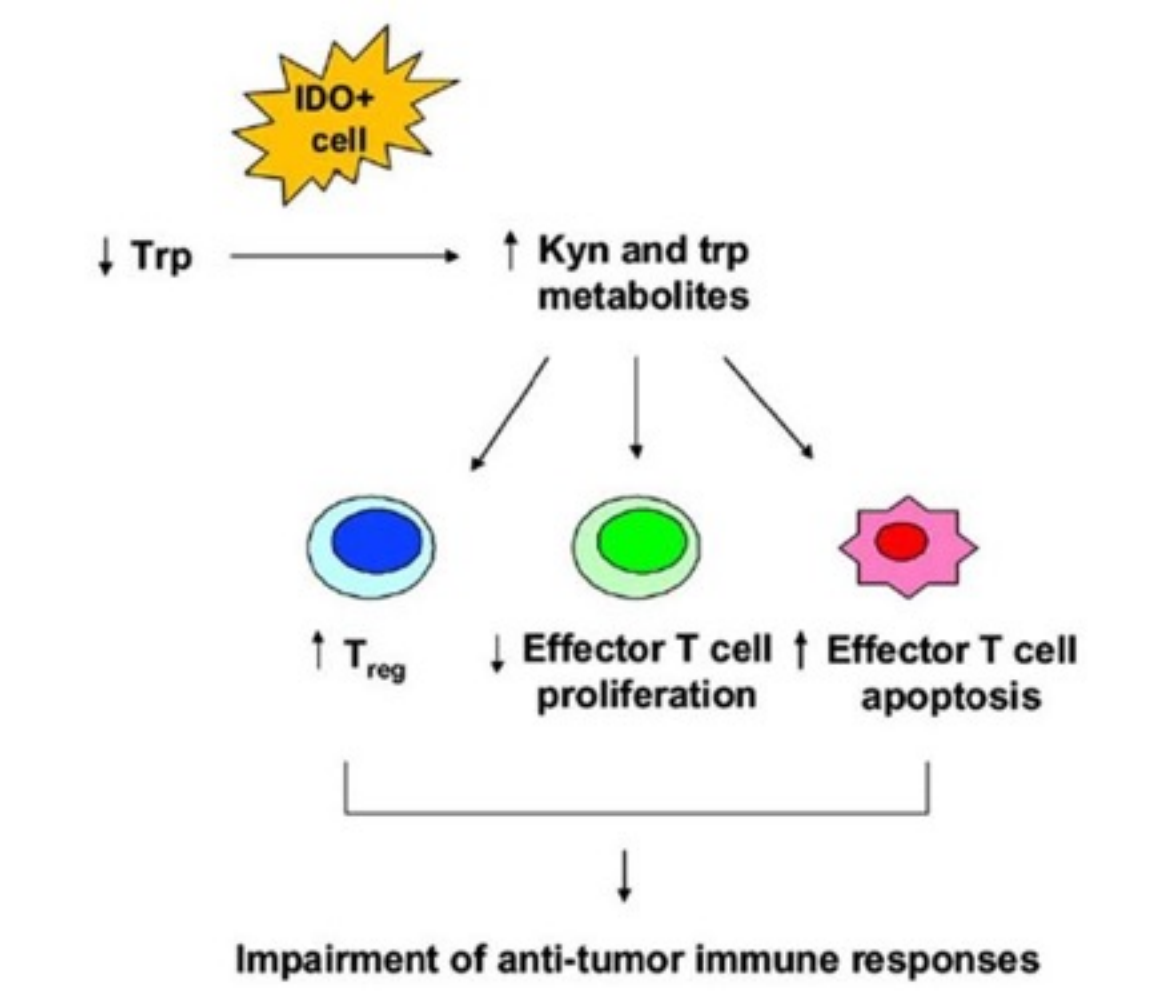
Indolemine 2,3 dioxygenase (IDO)



Mechanism of IDO gene regulation

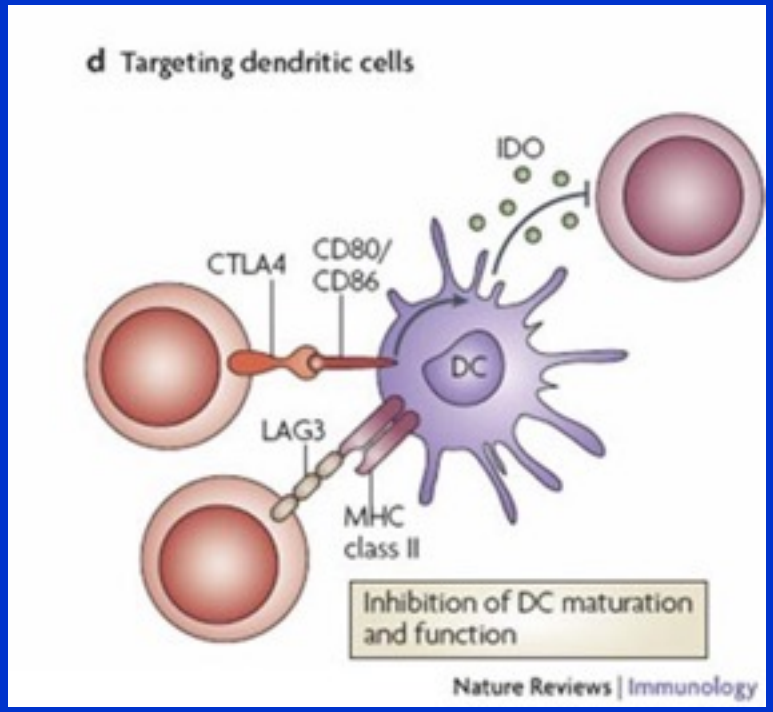
[9]Immun Rev 2008; 222: 206-221.

Indolemine 2,3 dioxygenase (IDO)



[9]Immun Rev 2008; 222: 206-221.

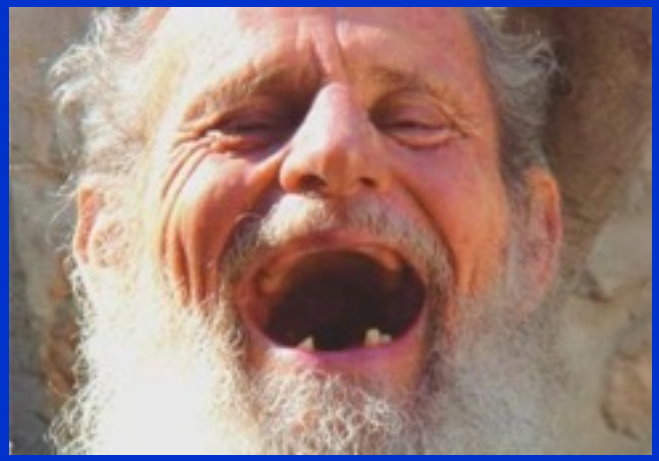
Feed forward mechanism – Treg ←→ DC

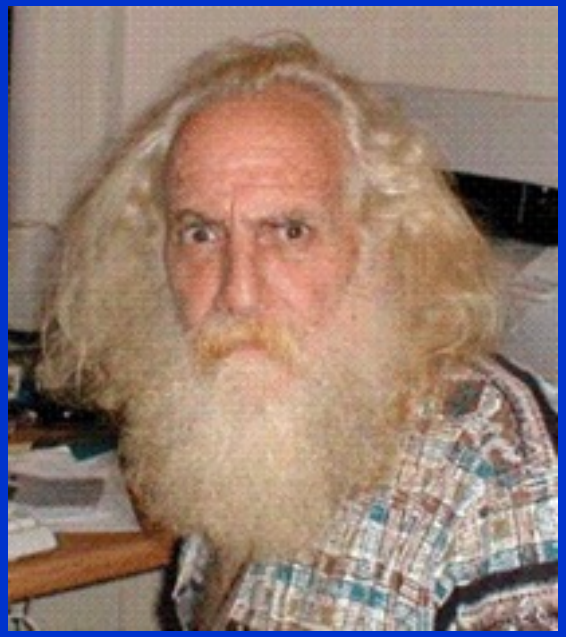


- typically CD4(+)CD25(+)FOXP3(+)
- can be antigen specific
- mediated via IDO to affect other T cells and APC
- depletion prior to AT has proven efficacy

Senescence

Insufficient numbers/persistence of transferred cells

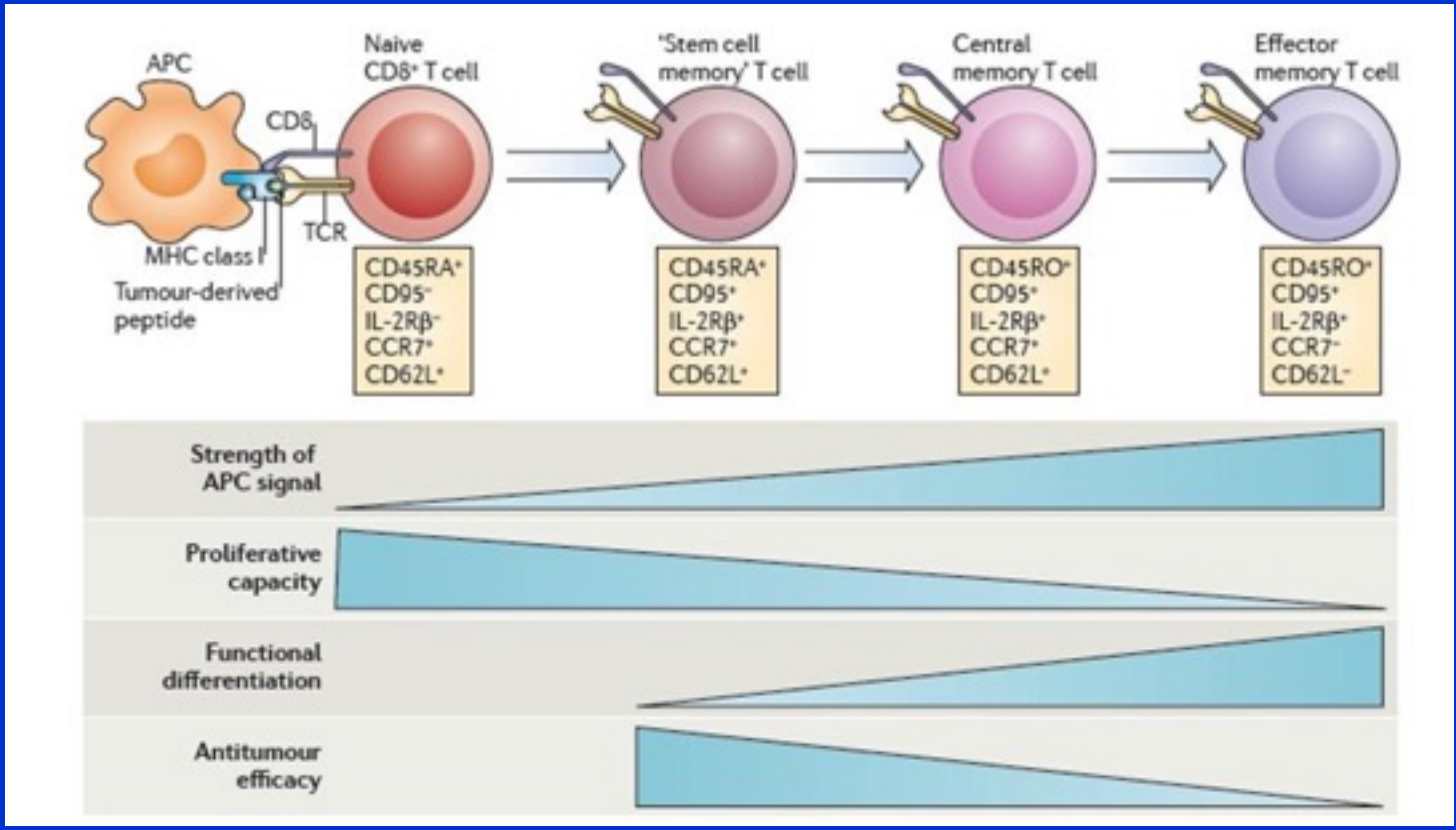




- T cells close to senescence by time of adoptive transfer

[11]J Immunother. 2011; 3(3): 407-421.

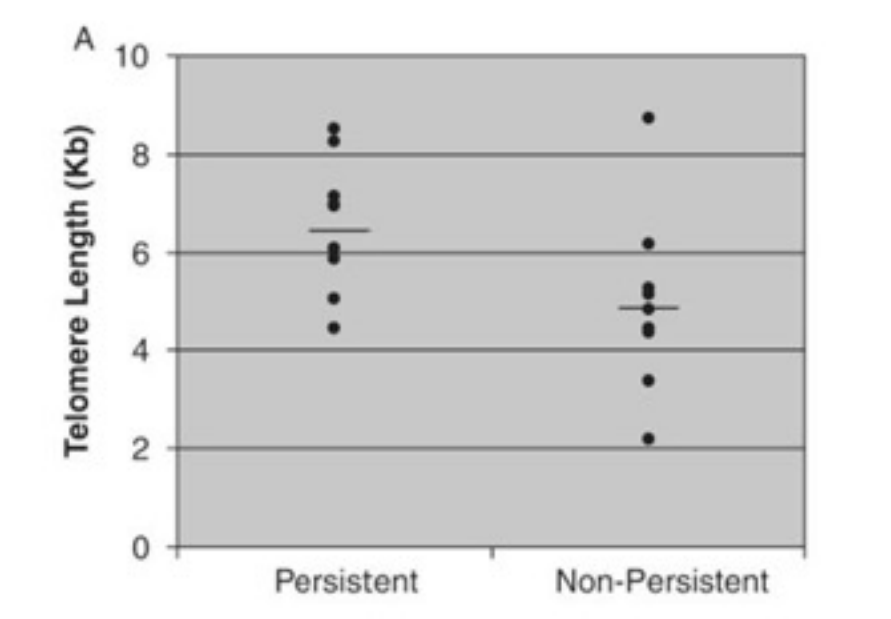
Insufficient numbers/persistence of transferred cells



- less differentiated "younger" better?

[11]J Immunother. 2011; 3(3): 407-421.

Insufficient numbers/persistence of transferred cells



- Persistence in vivo beneficial
- Telomere regulation via "shelterin"
- control telomerase activity → "immortal" T cell?

[11]J Immunother. 2007; 30(1): 123-129.

Limited trafficking into tumor

Limited lymphocyte trafficking to tumor

- cell mediated killing requires direct contact
- tumor vessels are grossly abnormal
- normal physiologic mechanisms of lymphocyte trafficking nearly impossible

Abnormal tumor vasculature as means of immune escape

Healthy vessel	Tumor vessel
	
Well organized Defined arterioles and venules Regularly distributed Non-dilated Non-permeable Mature and coated with mural cells Low interstitial pressure Complete basement membrane Endothelial cell and mural cell Appropriate expression of markers Normal rate of blood flow	Disorganized Undefined arterioles and venules Unevenly distributed Dilated Highly permeable Premature and lack of mural cells High interstitial pressure Lack basement membrane Mosaic cells High or low expression of markers Sluggish blood flow

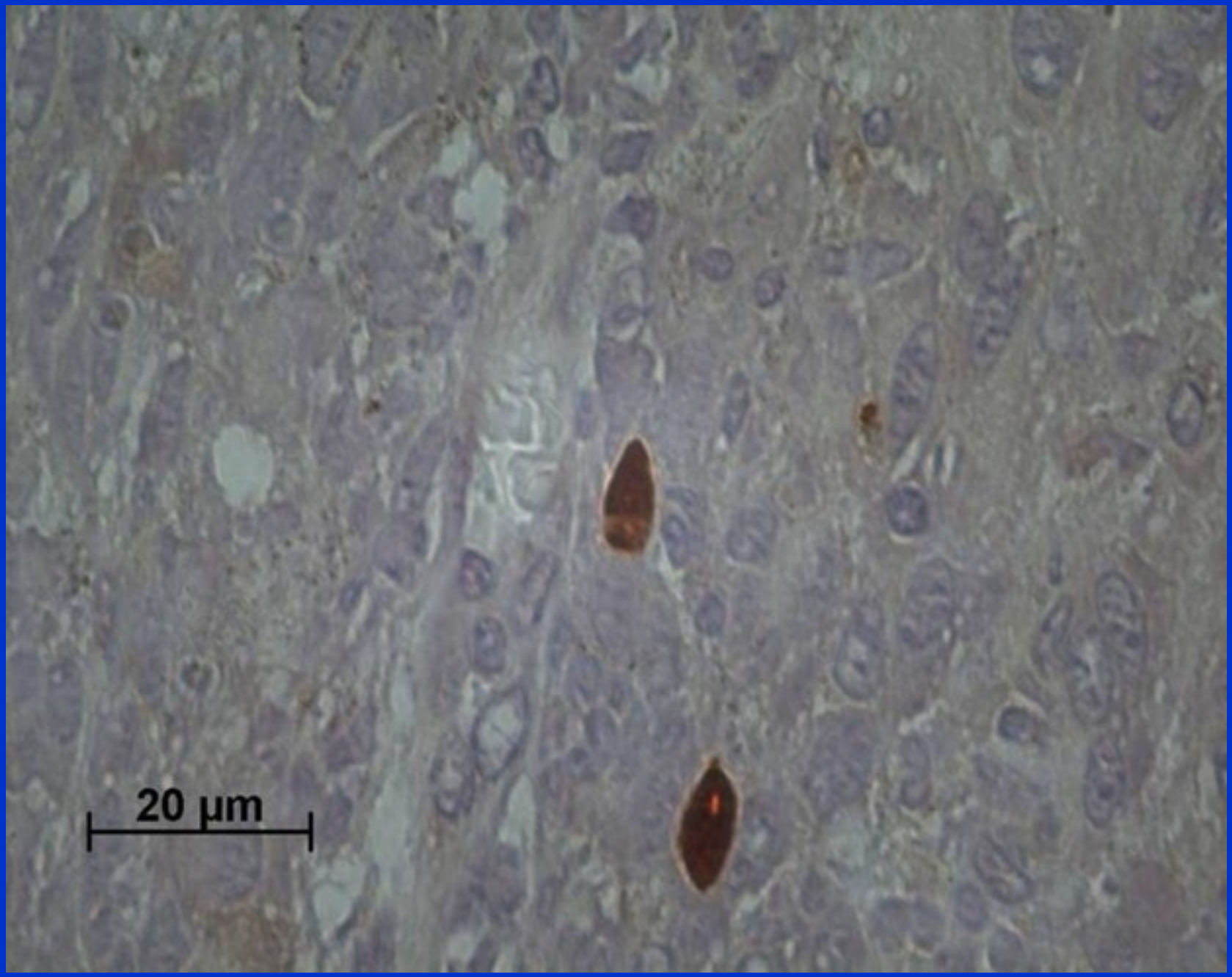
Lymphocyte trafficking in preclinical models

Current models of trafficking of lymphocytes

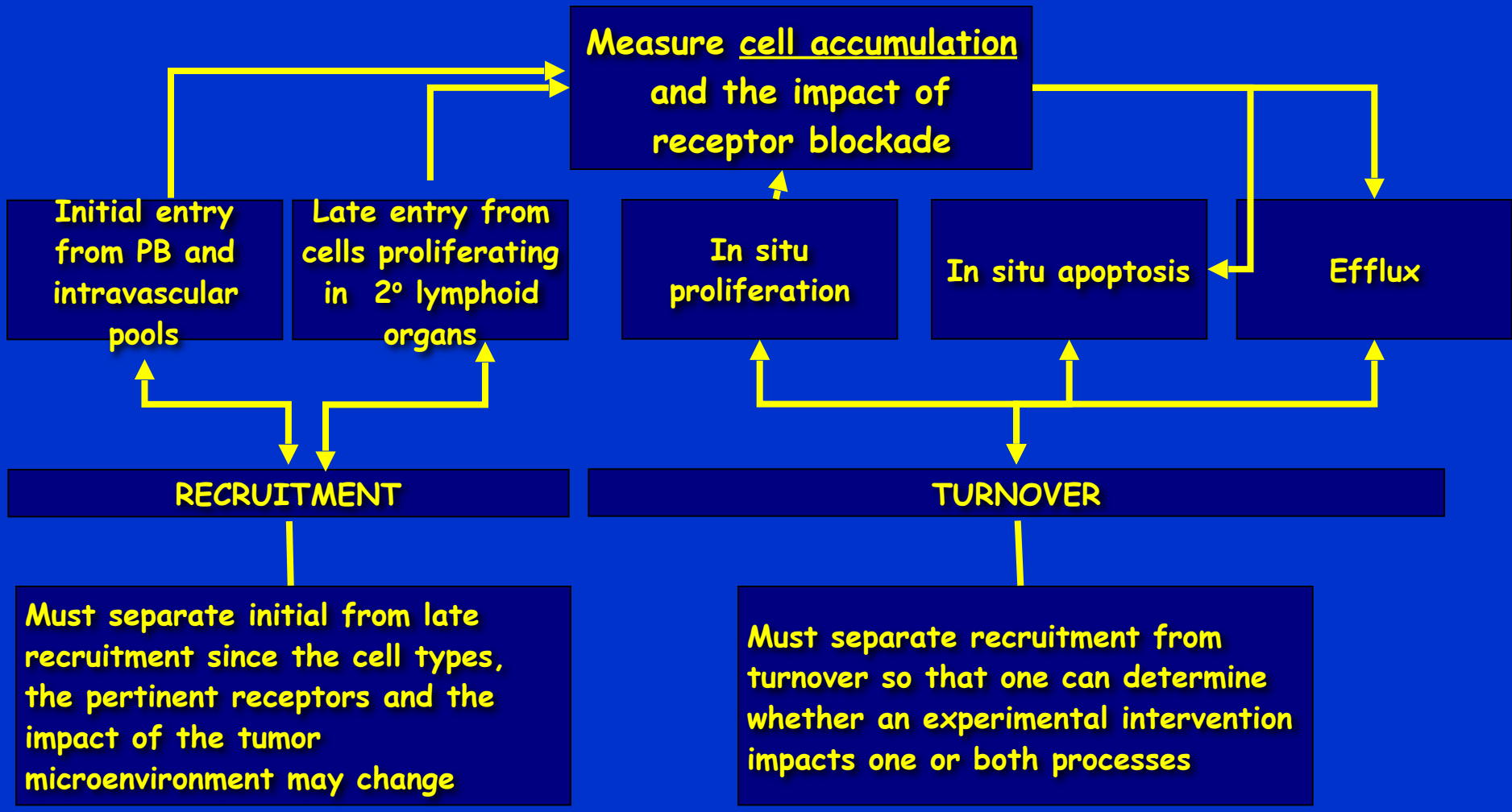
selectins → chemokine receptors → integrins

Methods of following lymphocyte movement -

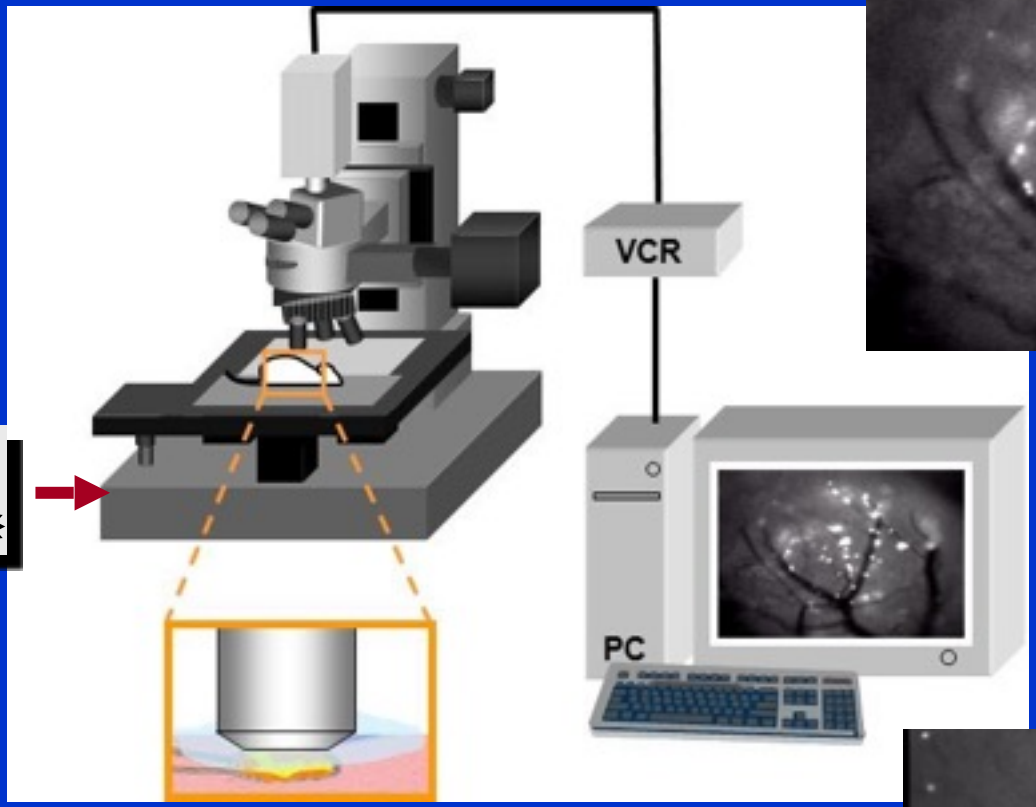
radiolabeled cells
cell surface marker variation
fluorescent tracking dyes
genetic variations

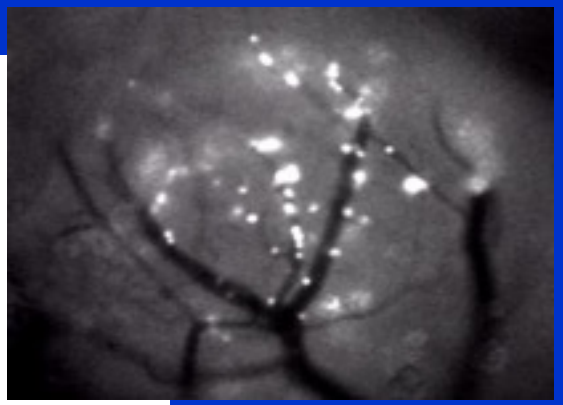

20 µm

How does one study T-cell trafficking in vivo?

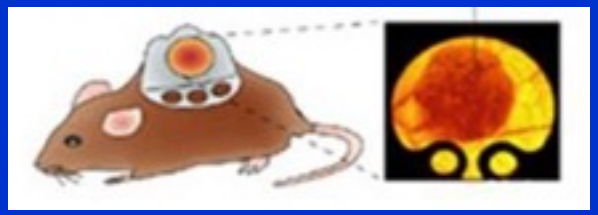


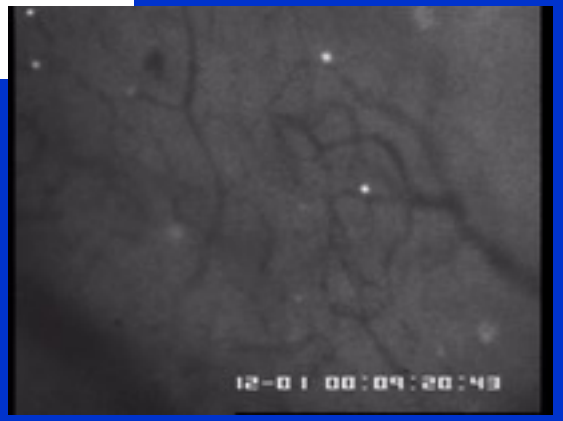
Intravital microscopy (IVM)





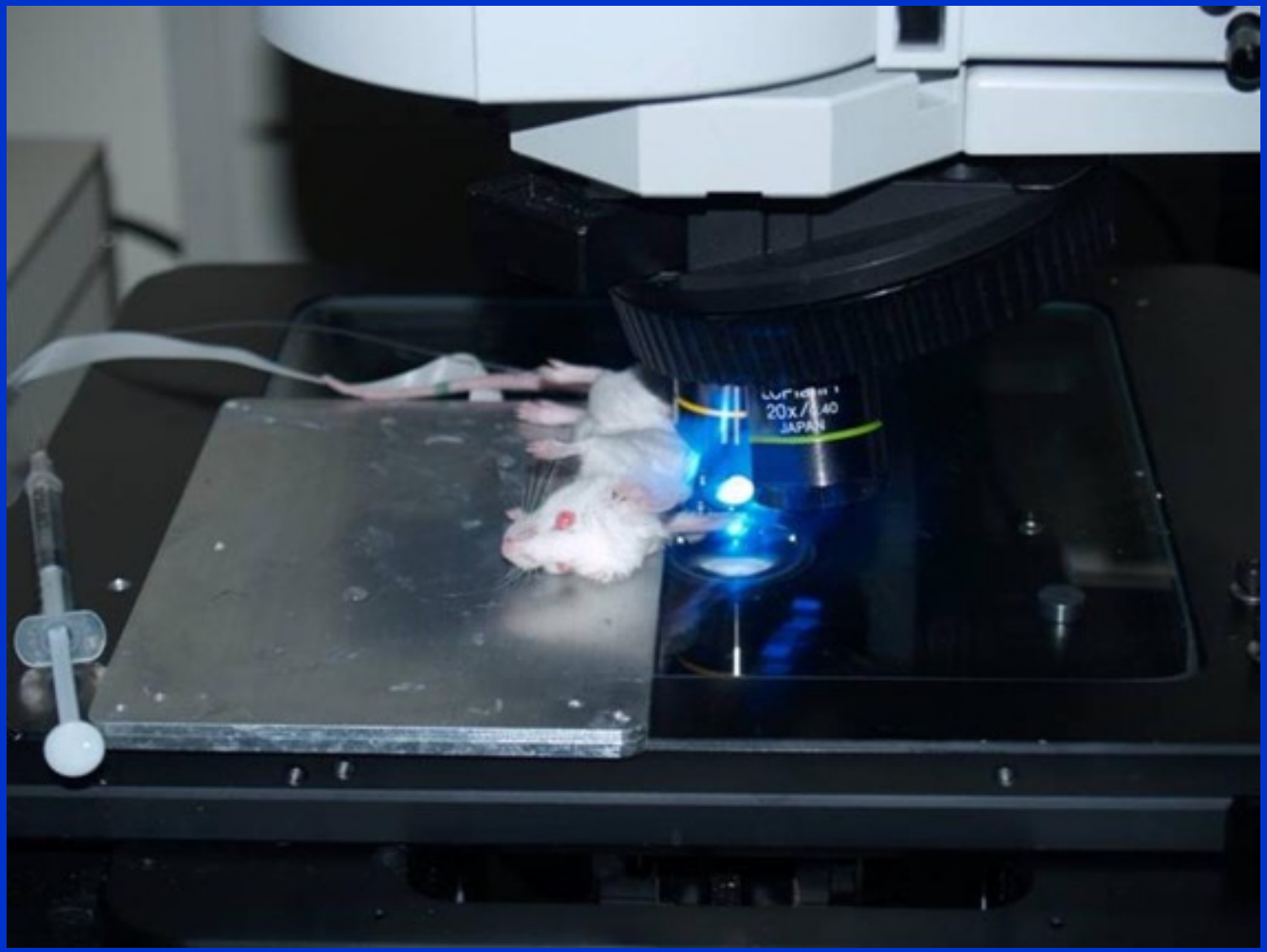
Inject calcein-labeled cells



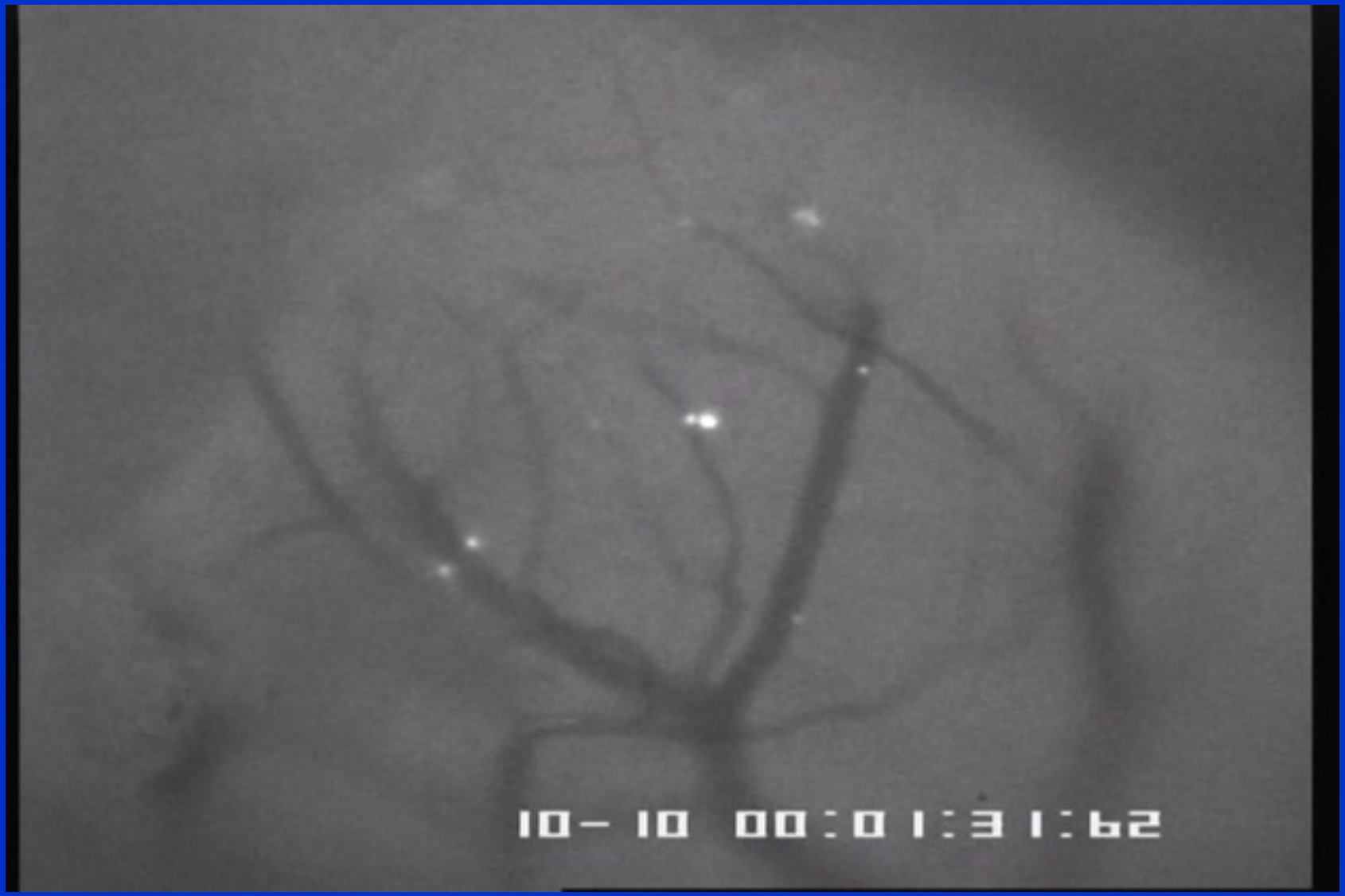


Intravital microscopy (IVM)

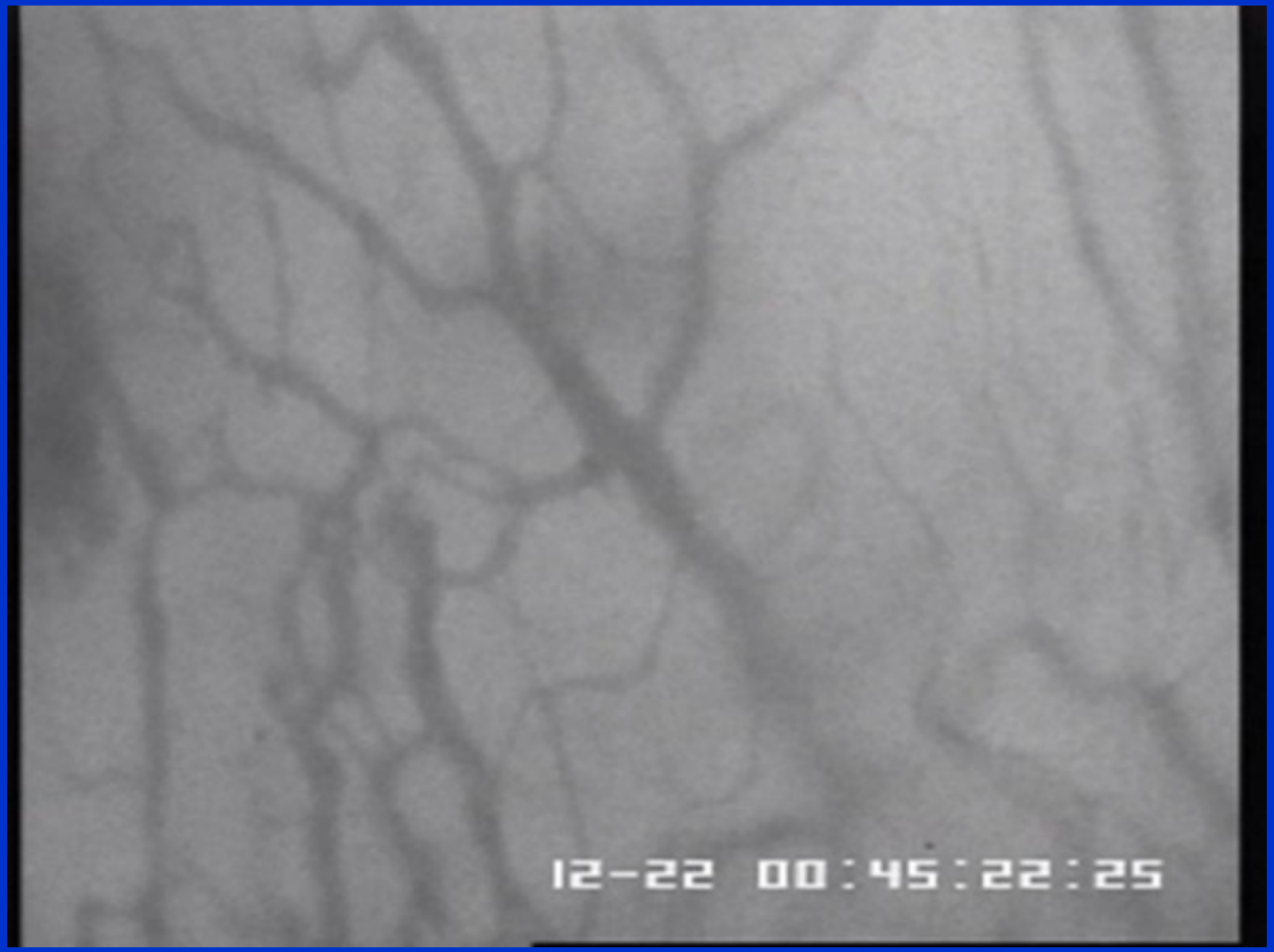
Intravital microscopy (IVM)



Intravital microscopy (IVM) lymph node



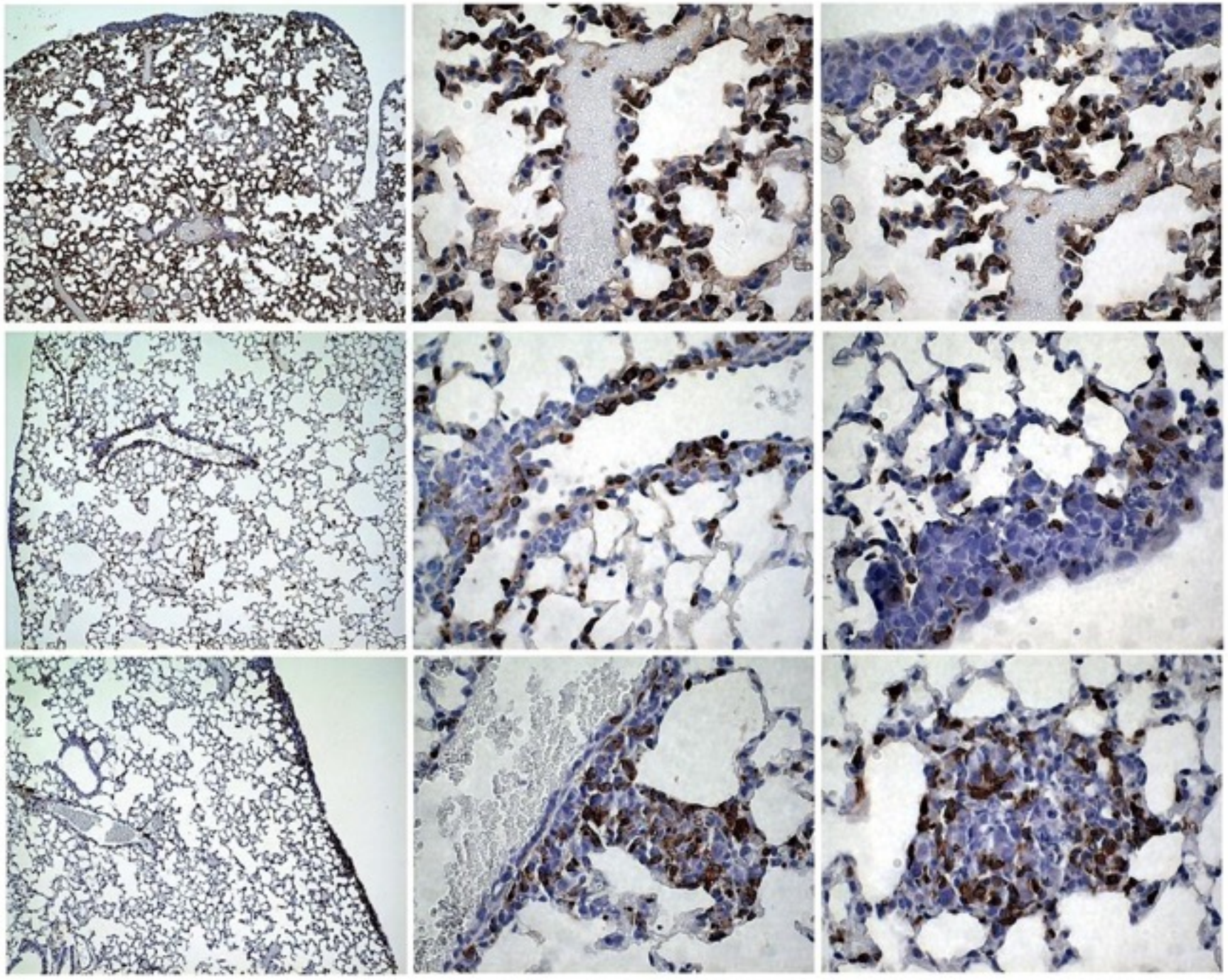
Intravital microscopy (IVM) tumor



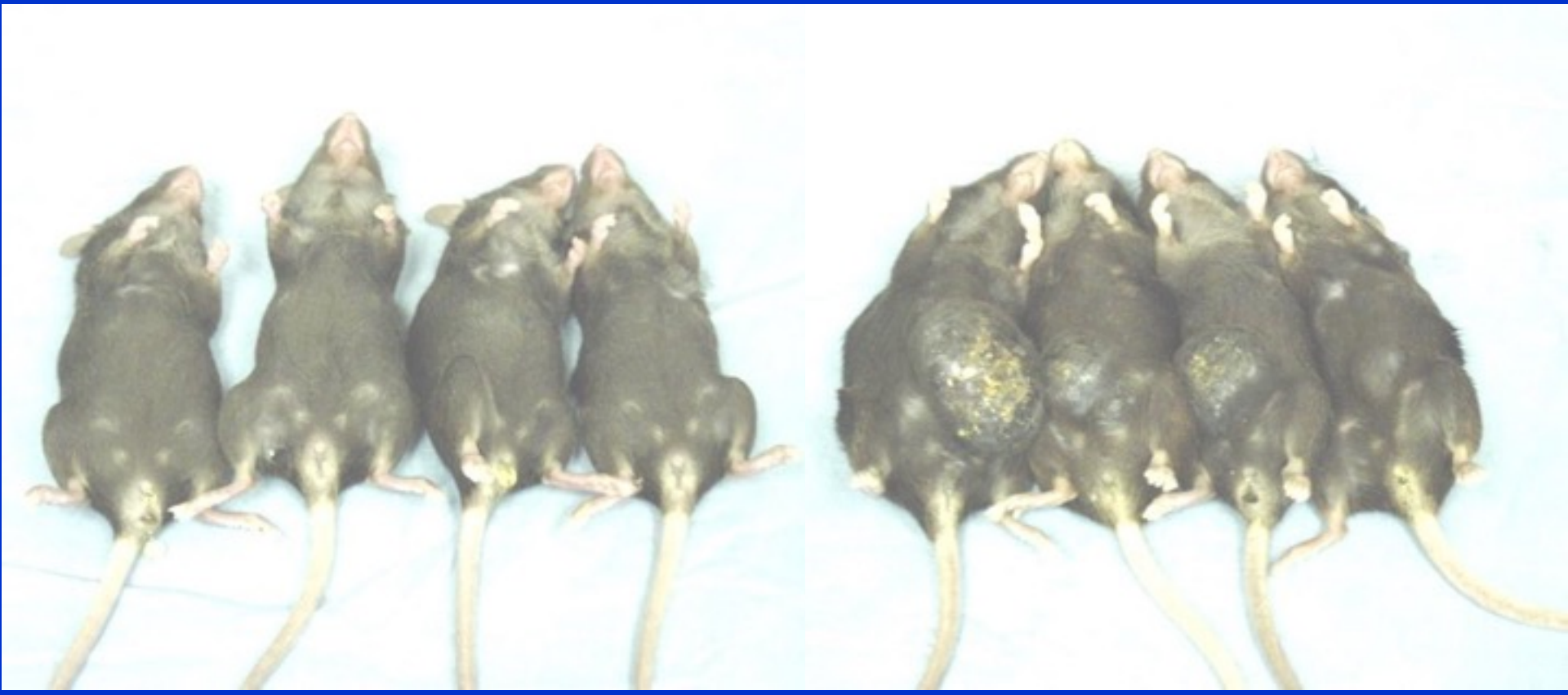
1hr

4hr

24hr



A picture is worth a thousand graphs . . .



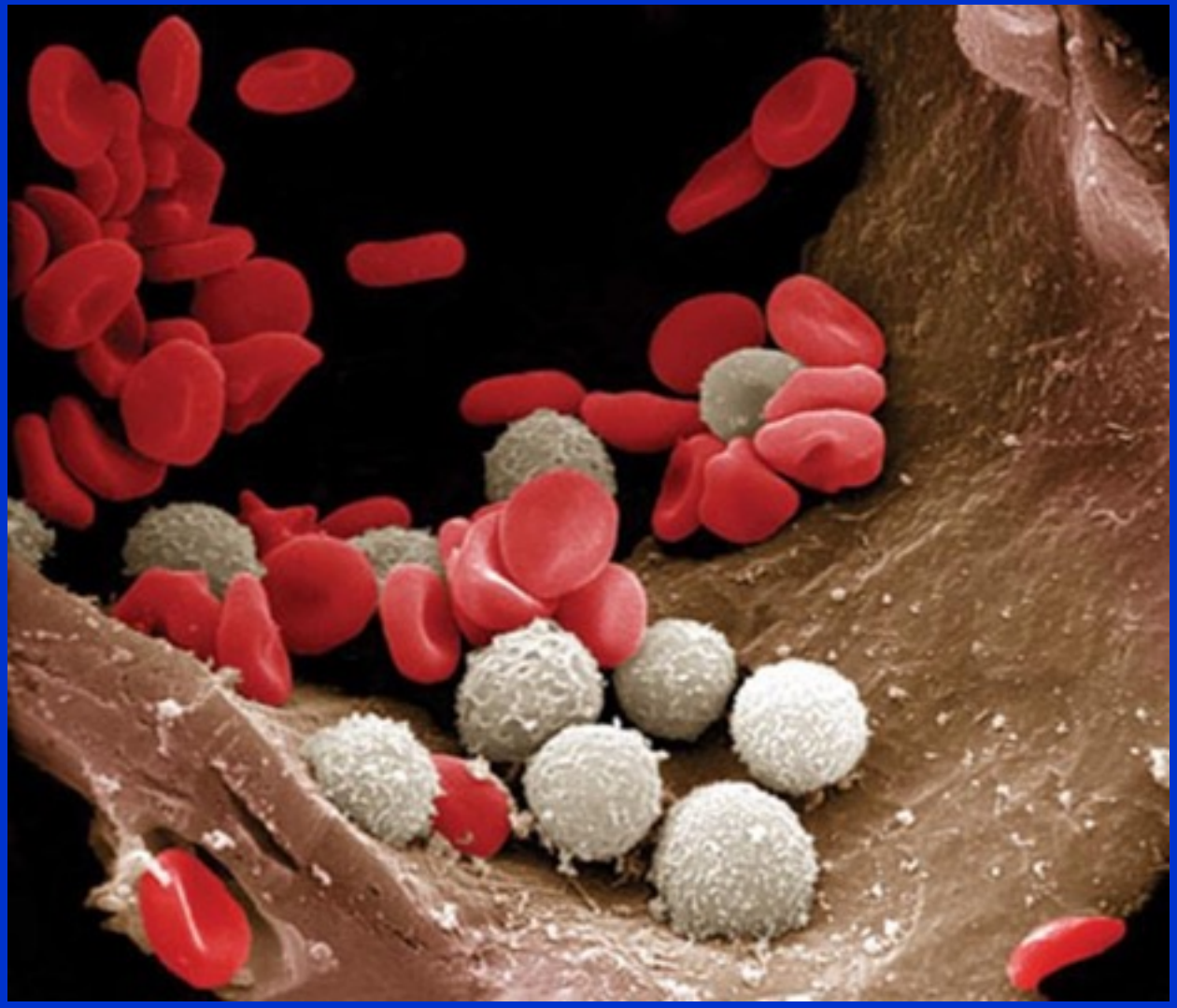
wild type

PE-KO mice

Why does adoptive immunotherapy fail?

- poor antigen display by tumor
- antigen loss
- hostile tumor microenvironment
- immune tolerance
- regulatory T cells
- insufficient numbers/persistence of cells
- inability to access tumors – limited lymphocyte trafficking

Questions?



Suggested reading

Rosenberg SA et al. Cell transfer therapy for cancer: lessons from sequential treatments of a patient with metastatic melanoma. J Immunother. 2003; 26(5): 385-393.

Ohta A et al. A2A adenosine receptor protects tumors from antitumor T cells. PNAS. 2006; 103(35):13132-13137.

Katz JB et al. Indoleamine 2,3-dioxygenase in T-cell tolerance and tumoral immune escape. Immunological Reviews. 2008; 222:206-221.

Gattinoni L et al. Adoptive immunotherapy for cancer: building on success. Nat Rev Immunol. 2006; 6(5):383-393.